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ARTICLES

RESIDUAL TOXICITY OF SOME NEW INSECTICIDES TO ADULTS OF *ANOPHELES QUADRIMACULATUS* SAY

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This paper is one in a series presenting the results obtained in a program to develop new insecticides as residual sprays for the control of adult mosquitoes. In previous papers LaBrecque *et al.* (1958, 1960), Gahan and Hoffman (1959), and Gahan *et al.* (1961) showed malathion and *m*-isopropylphenyl methylcarbamate (Hercules AC-5727) were sufficiently effective to justify extensive testing in infested areas where the chlorinated hydrocarbon insecticides have failed to give satisfactory control. The present report includes data obtained in the laboratory with five new compounds and in naturally infested buildings with four of the compounds mentioned in previous papers cited above.

LABORATORY TESTS. In the laboratory evaluations, acetone solutions of the compounds were sprayed on plywood surfaces at one or more dosages. At intervals after treatment approximately 50 adults of *Anopheles quadrimaculatus* Say (mixed sexes) were exposed on the treated surfaces under half sections of Petri dishes for periods of 1, 5, 15, 30, or 60 minutes. All the exposures were not used each time but an effort was made to achieve a range of mortalities that reached 100 percent. After exposure, the insects were transferred to untreated screen holding cages, furnished cotton that had been saturated with 10 percent honey solution, and held for 24-hour mortality counts.

The strain of *A. quadrimaculatus* used in these tests has developed a high level of resistance to dieldrin in the course of many years of laboratory maintenance. At the time of these tests the larvae had about 100-fold resistance and adult mortality did not exceed 16 percent with the W. H. O. standard test kit following a 1-hour exposure on papers treated with 0.8-percent dieldrin.

One of the compounds, *o*-isopropoxyphenyl methylcarbamate (Bayer 39007), was closely related to the *m*-isopropylphenyl methylcarbamate (Hercules AC-5727) that was highly effective in previous studies. It is a white crystalline material that forms an almost clear solution in acetone. When applied to plywood, it did not stain and had only a slight odor. The other compounds are esters of phosphorothioic or phosphinothioic acids. *O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate (Bayer 41831) is a brown liquid with a mild unpleasant odor. It gave a yellow solution in acetone and stained the plywood boards very slightly. *O,O*-diethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37341) and *O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37342) are both colorless liquids that do not stain. *O*-(4-methylthio-*m*-tolyl) dimethylphosphinothioate (Bayer 34098) is a white powder with a mild unpleasant odor. It forms a colorless solution in acetone and does not stain. DDT, malathion, and Hercules AC-5727 were included in these tests as standards.

The minimum exposure times required to produce between 90 percent and 100 percent mortality within 24 hours after the residues had aged various periods of time are presented in Table 1.

Bayer 39007 and Bayer 41831 were the compounds of greatest interest. When they were used at dosages of 100 mg. per square foot, exposures as short as 1 to 5

TABLE 1.—Minimum exposure times required to produce at least 90-percent mortality within 24 hours among *Anopheles quadrimaculatus* exposed on plywood panels treated with various insecticides and aged for various periods

Insecticide	Dosage (mg./sq. ft.)	Minutes needed after aging residues— 1 wk.	2 wk.	4 wk.	8 wk.	12 wk.	16 wk.	20 wk.	24 wk.	32 wk.
Bayer 39007	100	1	5	1	5	15	15	1	30	5
	10	5	15	15	60	30	>60	..	..	..
	1	60	30	30	>60	60	>60	..	..	..
Bayer 41831	100	5	5	5	5	15	30	30	30	15
	10	60	30	60	15	>60	..	..	..	..
Bayer 37341	100	60	60	60	>60	..	..	..	..	..
Bayer 37342	100	30	30	30	60	60	60	60	>60	..
Bayer 34098	100	60	30	>60	..	..	..	..	..	..
Hercules	100	1	1	1	1	5	5	15	15	15
AC-5727	10	5	15	15	15	30	30	30	30	60
	1	15	60	30	30	60	30	60	30	60
Malathion	100	30	60	30	15	60	30	15	30	15
		15	15	30	15	30	15	30	30	..
DDT	100	30	30	15	15	15	60	30	60	..

minutes killed above 90 percent of the insects during the first 2 months of aging. Throughout this same period DDT and malathion needed exposures of 15 to 30 minutes to produce similar results. By the time the residues were 12 to 32 weeks old Bayer 39007 was still better than malathion or DDT, and Bayer 41831 was about as effective as malathion, but superior to DDT. Bayer 39007 was somewhat less effective than Hercules 5727, especially at dosages of 1 and 10 mg. per square foot. At 10 mg. per square foot both these compounds were better than Bayer 41831.

Bayer 39007 is another fast-acting compound like Hercules AC-5727. On 7-day-old residues of 100 mg. per square foot, exposures for only 5 minutes caused 100 percent knockdown before the mosquitoes could be removed from the boards. Exposures as short as 1 minute produced 100 percent knockdown within 30 minutes. Bayer 41831 was not as rapid in action, for the knockdown in 30 minutes was 0 percent after a 5-minute exposure and only 37 percent after a 30-minute exposure. Fast action is not a necessary characteristic of compounds for use in malaria eradication programs, but it is highly desirable so that mosquito annoyance can be reduced as rapidly as possible.

Bayer 37341, Bayer 37342, and Bayer 34098 at dosages of 100 mg. per square foot required exposures of 30 to 60 minutes to cause mortality above 90 percent the first week after treatment, and the residues were less durable than those of the previously mentioned compounds. None caused any 30-minute knockdown after 30 minutes of continuous exposure, nor were they highly toxic at dosages of 10 mg. per square foot.

Toxicological information received from the manufacturers indicated that at least three of the compounds probably would be safe to use in actual control work. The oral toxicity to warm-blooded animals based on an LD-50 in mgs./kg. of body weight was 95–200 mg. to rats for Bayer 39007 [1] and 1180 mg. to mice for Bayer 37342. The latter compound was not toxic to mice at 500 mg. The status of Bayer 41831 is not clear but it appears to be no more toxic than Bayer 39007. No information was available on the toxicity of Bayer 37341 or Bayer 34098.

[1] Information on this compound received from Chemagro Corporation, based on data collected by K. P. DuBois, Department of Pharmacology, University of Chicago, as well as the Farbenfabriken Bayer Company, Elberfeld, West Germany.

Field Tests. Four compounds that were previously reported to be effective in laboratory tests by LaBrecque *et al.* (1960) or Gahan *et al.* (1961) were tested in buildings naturally infested with *Anopheles quadrimaculatus*. A dog house treated with a malathion standard was built of concrete blocks, but the other buildings were made of wood. All buildings were located in a swampy area around Bushnell, Sumter County, Florida, where cattle, pigs, or chickens were plentiful. Two series of tests were made; in the first series the applications were made on February 10, 1960, and in the second on July 13, 1960. Hercules AC-5727 was applied at 100 mg. per square foot in a calf barn in series 1 and in a small tool shed in series 2, and at 50 mg. in a small pump house in series 1. Dimethoate, Methyl Trithion (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate), and Sevin (1-naphthyl *N*-methyl carbamate) were applied to two buildings each at 100 mg. per square foot in series 2. Animal barns were used for the dimethoate and one of the Methyl Trithion treatments. The other Methyl Trithion application was made in an old deserted building with broken windows and doors through which the mosquitoes could enter. Two privies at a rural church were sprayed with Sevin. In each series DDT and malathion were used at 100 mg. per square foot as standards. One of the buildings treated with malathion was a tractor shed on a farm that had cattle, pigs, and chickens; the other buildings regularly housed animals. All residues were applied as water emulsions. Hercules AC-5727, Methyl Trithion, Sevin, and DDT sprays were prepared from emulsion concentrates containing 25 percent insecticide, 65 percent xylene, and 10 percent Triton X-100. The malathion and dimethoate concentrates were formulated by their manufacturers.

Pre- and post-treatment counts were made of the *Anopheles* mosquitoes resting in the treated buildings as well as in eight untreated buildings, four of which were used as checks in series 1 and four in series 2. Pre-treatment counts were made on at least two occasions within 1 month of the spray applications and all counting was done as near the middle of the day as practicable. Averages of the counts made before treatment and the percentage reduction in the mosquito population at intervals after treatment are presented in Table 2. A test was completed as soon as the percentage reduction became less than 90 percent.

Hercules AC-5727 at 100 mg. per square foot eliminated all the mosquitoes from the treated buildings during the first 9 to 11 weeks and the control remained above 90 percent for at least 25 to 28 weeks. The test in the building under observation for 25 weeks was still highly effective when this manuscript was prepared and the final answer on its durability was not available at that time. Effective control deteriorated more rapidly with this compound at the 50-mg. dosage for although it also eliminated all mosquitoes for 9 weeks it was not highly effective after 11 weeks.

Incomplete information on its safety obscures the future of this compound in mosquito control campaigns at the present time.

Some mosquitoes were present on almost every occasion in the buildings sprayed with dimethoate, Methyl Trithion, and Sevin, and control deteriorated rapidly. In addition to those counted on the walls and ceilings in these buildings, mosquitoes that could not be counted were flying in the air. These compounds appeared to be too slow acting to be of much value.

The malathion standard applied in the dog house constructed of cement blocks caused less than 90 percent reduction as early as 6 to 7 weeks after treatment, but this insecticide remained highly effective for 9 weeks in the wooden tractor shed. The DDT standard was almost completely effective, with control above 99 percent, for at least 25 to 36 weeks.

The *Anopheles* mosquitoes in this part of Central Florida obviously were not highly resistant to DDT. In studies conducted during 1958 in Arkansas by Gahan

TABLE 2.—Effect of insecticides on mosquito densities in naturally infested buildings. (Pump house treated at 50 mg. per square foot, all other applications at 100 mg. per square foot.)

Type of building	Pre-treatment count	Percent reduction [a] at indicated number of weeks following treatment											
		1–2	3–4	6–7	9	11	14	18	25	28	32	36	40
Series 1													
						Hercules AC-5727							
Calf barn	427	100	100	100	100	96	92	100	99.9	97	88	..	..
Pump house	53	100	100	100	100	50	..	..	..	..	..	..	..
						Malathion							
Tractor shed	232	100	99.8	99	99	64	..	..	..	..	..	..	..
						DDT							
Animal shed	153	100	100	100	99	100	100	99	99	99.7	100	97	89
						Untreated							
Storage shed	34	60	52	81	3	+102	+276	+18	60	59	31	+29	+241
Cow shed	489	68	62	91	+37	+513	+513	+513	+464	+473	+104	+474	+413
Chicken house	18	+42	+208	5	+164	+1317	+1150	+784	+544	+399	+811	+236	+287
Privy	20	53	70	60	25	+473	+433	+125	90	+95	+165	+135	+55
Series 2													
						Hercules AC-5727							
Tool shed	62	100	100	100	..	100	98	98	94	..	..	..	..
						Dimethoate							
Animal barn	244	97	99	91	..	74	..	..	..	..	..	..	..
Animal barn	452	99	99	97	..	85	..	..	..	..	..	..	..
						Methyl Trithion							
Deserted building	202	96	98	70	..	..	..	..	..	..	..	..	..
Animal barn	86	33	..	..	..	..	..	..	..	..	..	..	..
						Sevin							
Privy	120	99	98	47	..	..	..	..	..	..	..	..	..
Privy	103	99	100	73	..	..	..	..	..	..	..	..	..
						Malathion							
Dog house	36	100	90	60	..	..	..	..	..	..	..	..	..

TABLE 2.—Continued

Type of building	Pre-treatment count	Percent reduction [a] at indicated number of weeks following treatment											
		1–2	3–4	6–7	9	11	14	18	25	28	32	36	40
						DDT							
Animal barn	410	99.7	99.9	99.8	..	99.9	100	100	100	..	..	..	..
						Untreated							
Storage shed	72	46	74	45	..	49	46	56	..	..	..	..	..
Tractor shed	375	+60	..	..	..	..	+60	+33	..	..	..	..	..
Garage	172	40	71	71	..	79	55	80	..	..	..	..	..
Roof shelter	47	+28	26	+527	..	+538	+332	+318	..	..	..	..	..

[a] Plus signs indicate percent increase above the pretreatment count.

and Hoffman (1959), DDT caused above 90 percent reduction for only 2 to 11 weeks in infested buildings treated at 200 mg. per square foot. In the present series of tests treatments applied at half that dosage remained effective more than twice as long as the most effective application made in Arkansas. No malaria eradiction program has been conducted recently in this part of Florida and the farmers do not apply insecticides to their crops as frequently as in Arkansas so the mosquitoes probably contact surfaces treated with DDT less frequently.

SUMMARY. Residual spray tests conducted in the laboratory showed *o*-isopropoxyphenyl methylcarbamate (Bayer 39007) and *O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate (Bayer 41831) to be highly effective against adults of *Anopheles quadrimaculatus*. At dosages of 100 mg. per square foot they were superior to the DDT and malathion standards during the first 2 months of aging and exposures as short as 1 to 5 minutes were sufficient to produce above 90 percent mortality. After 16 to 32 weeks Bayer 39007 was the best of these four materials and Bayer 41831 was about as effective as malathion, but superior to DDT. Bayer 39007 was somewhat less effective than a closely-related compound, *m*-isopropylphenyl methylcarbamate, (Hercules AC-5727) which was used as a standard. Both these compounds were fast acting; 5-minute exposures on 7-day-old applications produced 100-percent knockdown before the mosquitoes could be removed from the treated surfaces. *O,O*-Diethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37341), *O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37342), and *O*-(4-methylthio-*m*-tolyl) dimethylphosphinothioate (Bayer 34098) also were effective, but were less toxic and durable than the other compounds.

Four compounds were evaluated as 100-mg.-per-square-foot residues in buildings naturally infested with adults of *Anopheles quadrimaculatus*. Hercules AC-5727 at 100 mg. per square foot eliminated all the mosquitoes from treated buildings during the first 9 to 11 weeks and produced control above 90 percent for at least 25 to 28 weeks. Dimethoate, Methyl Trithion (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate), and Sevin (1-naphthyl *N*-methyl carbamate) failed to eliminate all *Anopheles* mosquitoes from treated buildings as fresh applications and control deteriorated rapidly.

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SOME DATA ON THE DOMESTICITY OF *AEDES SCAPULARIS* (RONDANI) IN SÃO PAULO, BRAZIL[1]

O. P. FORATTINI

Recent investigations show the increasing importance and interest of *Aedes scapularis* as a potential vector of pathogenic organisms to both man and animals. Up to the present, very little is known concerning its behaviour towards humans. Only few cases of anthropophily and, occasionally, its frequency in human dwellings have been recorded. Lane (1936) made several collections in Matto Grosso, Brazil, and found only one specimen inside houses. He thought that this behaviour was merely accidental. Kumm and Zúniga (1942), in El Salvador, report the finding of this species inside houses, without giving additional data. Rachou *et al.* (1955), making regular collections at Santa Catarina, Brazil, never found it inside houses, but outdoors found a man hour collecting average of 9.5. One can see, therefore, that information regarding the behaviour of this mosquito is very scarce.

During the course of investigations on the density of anophelines of the subgenus *Kerteszia,* specimens of *Aedes scapularis* were regularly collected. These investigations were made at the localities of Cananeia, Casa Grande and Boraceia, in the State of São Paulo, Brazil. The results obtained permit us to give some data on the frequency of this culicid in human dwellings.

The collections were made indoors and outdoors simultaneously, two or three times a week, starting at sunset and ending at 21:00 o'clock. For this work, men were employed who had been previously trained to collect mosquitoes on themselves, on walls and on other objects.

Cananeia is situated in the south coastal region of the State of São Paulo and is actually an active malaria transmission focus. The town is very old and the surrounding vegetation has suffered constantly from man's destruction. The other two localities are non-malarigenous. Casa Grande is an established population nucleus, founded some decades ago. Boraceia is situated about ten kilometers from the former and contains only a few houses, which are very close to the surrounding forest. We can conclude, therefore, that man's influence was more intensive and of longer duration at Cananeia, less so at Casa Grande and almost nonexistent at Boraceia.

The collections were made from November 1960 to March 1961 inclusive, so that the hot and rainy season of the year was included. The results obtained expressed as man-hour collecting averages, are shown in Table 1. Very remarkable differences were found. The greatest indoor frequency was found at Cananeia, a lesser one at Casa Grande and a negative one at Boraceia. Similar results were obtained concerning outdoor frequency.

There are reasons to believe therefore that *Aedes scapularis* is a mosquito with a tendency to adapt itself to the human environment. It is commonly found within the radius of human aggregations, preferring more open areas to intensely forested ones. In rural regions, its activity is usually concentrated around human dwellings. In that case, the results shown are suggestive. In Cananeia, an old, long inhabited town, there was more time for adaptation and the mosquito is often seen around human dwellings, invading them with some frequency. At Casa Grande, a more recently inhabited town, although firmly established, a similar observation is made but showing less intensity. Finally, at Boraceia, which is

[1] Contribution of the Department of Parasitology of the School of Hygiene and Public Health of the University of São Paulo, Brazil (Prof. J. O. Coutinho). This study was aided by the "Conselho Nacional de Pesquisas," Brazil.

TABLE 1.—Comparative man hours averages of *Aedes scapularis* mosquitos collected at three localities of the State of São Paulo, Brazil

Months	Cananeia		Casa Grande		Boraceia	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
1960						
November	1,0	5,7	0,3	1,2	..	0,3
December	1,3	6,8	0,4	1,7	..	1,1
1961						
January	1,2	4,3	0,7	2,8	..	1,2
February	1,1	3,2	0,3	2,8	..	1,1
March	1,4	4,0	0,3	2,4	..	0,5
Total	1,2	4,5	0,4	2,2	..	0,9

situated practically inside the jungle and where man plays a small part, there was very little frequency outdoors and none indoors.

Based on these observations, we conclude that *Aedes scapularis* is becoming progressively domesticated. Everything points to this tendency. Therefore, more attention needs to be paid to this species, considering that it is a potential vector of pathogenic organisms to man.

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TRAPS FOR DETERMINING DIRECTION OF FLIGHT OF INSECTS [1]

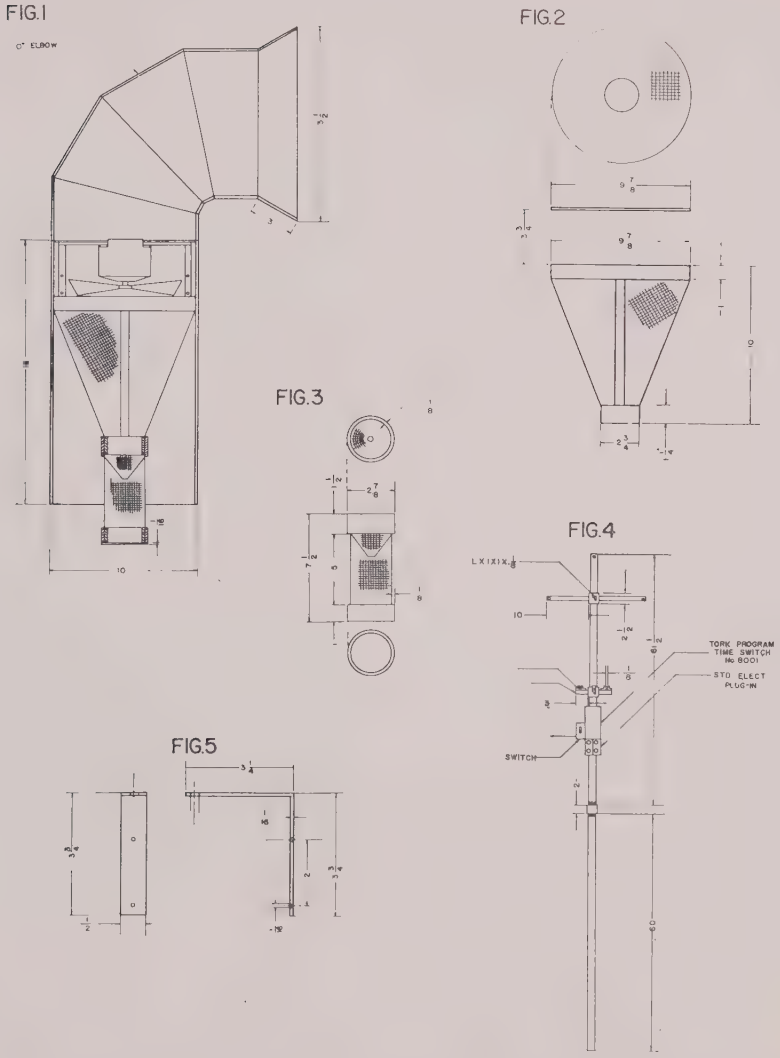
WILLIAM R. HORSFALL [2]
University of Illinois

Few means are available for determining the direction of movement of populations of insects in flight. Swarms and large insects may be seen and their direction may be traced. Small insects, dispersed populations and nocturnally active insects must be trapped. To this end nets have been rigged and sticky surfaces have been placed in the line of flight. Stationary nets reflect a bias in favor of movements down-wind. The sticky surfaces may create eddies that reflect unpredictable bias. The trap described herein is an active means for obtaining data on direction of movement of flying insects because once an insect is near the open face of a trap, it is sucked into the opening.

A battery of four traps comprises a single trapping unit (Fig. 6). The traps are held in place on a single center pole (Fig. 4) by four braces at the top and

[1] Funds for support of this project were provided by a grant from the National Institutes of Health, Washington, D. C.

[2] Grateful acknowledgment is made to Mr. Stewart Wilson and Roy Charles for aid in design and construction of the traps.



Directional Trap: Details of Construction.

Fig. 1.—Cut-away view of trap showing from top to bottom (1) elbowed intake, (2) fan, (3) concentrating cone and (4) collecting cage.

Fig. 2.—Concentrating cone with baffle screen (top and side view) and above.

Fig. 3.—Collecting cage (top, side and bottom view).

Fig. 4.—Supporting pole with timer and switch.

Fig. 5.—Detail of one post for support of fan.

four supports at the bottom of each. Straps between the upper braces keep the traps rigidly fixed. Each trap is essentially a cylinder with an elbow on top so that the intake is a flat face opening perpendicular to the ground. The four traps are so placed that each faces 90° from each adjacent one. In operation one faced toward each of the main points of the compass. The intake of each trap has an area of one square foot.

The trap has a body like the New Jersey trap in that it is a cylinder housing a fan, a concentrating cone and a collecting cage. It is different from the prototype in that it has an elbowed top expanded to an area of one square foot in cross section and has no light for attracting insects.

The body of the trap is a vertical cylinder of galvanized sheet metal 10″ in diameter (Fig. 1). Superimposed on it is a standard 10″ elbow to which has been soldered a cone flared from 10″ to an outside diameter of 13½″. The face of the intake is perpendicular to the ground. The screen concentrating cone is made of copper screen mesh (14 x 18) attached to a sheet-metal sleeve at the top and one at the bottom. The top sleeve fits tightly inside the cylinder at the level of the fan blades. The bottom sleeve provides a friction fit for the collecting cage.

The type of collecting cage may be varied according to its purpose. If live specimens are wanted, a cage of screen with a cone trap in the top is suggested (as illustrated in Fig. 3). Tight containers provided with toxicants may be used as well.

The fan may be of any standard make available at refrigeration or electrical supply stores. The blade should be at least 8″ in diameter. The down-draft created by it while in operation should be sufficient to draw into the trap insects flying within 6″ from the intake.

The traps were operated continuously night and day or were operated at stated intervals by means of a timer. The timer used permitted operation at any interval over 15 minutes for one or several times during the day. For the most part traps were operated from the onset of darkness until midnight. An associated light trap providing separate hourly collections showed that young female *Aedes vexans* were usually on the wing during this interval.

The traps have an active role in collecting passing insects because each is provided with a fan that passes air through it. The area of active suction for an air-borne body like a mosquito is 6″ from the center face of the intake. Insects flying in any direction other than toward a trap will usually pass out of range. To all intents, therefore, insects that are collected are moving in the direction of the intake and at its level although some casual wanderers flying by within the range of the suction will be collected also. The principal value of the fan is to draw into the intake insects like mosquitoes before they are able to veer away after once coming within the range.

FIG. 6.—A battery of directional traps at a collecting site.

Suction created at the face of the intake was fairly uniform according to records made with a small anemometer of the fan-type. Draft at the center position varied from a low reading of 7.2′ per second to a high of 10.1′ per second. When a wind of 7.1′ per second was blowing from the east, no effect was perceptible on the velocity of the air entering the trap regardless of its facing. Three traps directed toward the east at the time showed an intake velocity

at the center that varied from 7.2′ to 8.9′ per second while three others facing west showed intake velocities of 7.7′ to 10.1′ per second.

on a flood plain when mosquitoes were moving from it. The mosquitoes began moving north while they were still visible against the sky. The trap facing south

TABLE 1.—Incidence of young females of *Aedes vexans* caught by a battery of directional traps prior to midnight, Piatt County, Illinois, June-July, 1960

Wind velocity			Number of mosquitoes collected				Direction of approach
Relative		Ft/sec	North face	South face	East face	West face	
Moderate	SW-SE	8.8	2	7	8	2	South & East
	ENE	5.9	12	3	0	6	North & West
Low	E	4.4	6	7	2	6	Erratic
	SW-SE	3.0	2	6	5	12	Erratic
	SW-SE	1.5	11	15	6	28	Erratic
	N	1.5	4	27	7	14	South & West

Mosquitoes enter the traps differentially, as is shown in Table 1. During any one trapping interval one trap usually collects the majority of mosquitoes. For example, during four of the six nights when young females were flying in the area of the trap, one trap collected twice or more than twice as many mosquitoes as any other. Since this group of traps was located on high ground with sources of mosquitoes in all directions, the mosquitoes could approach from all sides. The records indicate that they approached without regard to velocity or direction of wind during the flight.[3]

The assumption that the traps reflect direction of flight was verified at a location

[3] Velocities and direction of wind were recorded automatically at the collecting site.

caught all of the mosquitoes during the short time the observations were being made.

However, if two adjacent traps collected approximately the same number, the flight was presumed to be from a direction between the two, thus southeasterly or otherwise as the case may be. The tendency toward directional differences was more marked when flights occurred while a moderate wind was blowing.

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WIND-TUNNEL TESTS WITH PROMISING INSECTICIDES AGAINST ADULT SALT-MARSH MOSQUITOES, *AEDES TAENIORHYNCHUS* (WIED.)

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INTRODUCTION. Salt-marsh mosquitoes, *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Wlk.), the principal pest mosquitoes along the coastal areas of Florida, were first reported to have developed resistance to DDT by Deonier *et al.* (1950) and Deonier and Gilbert (1950). Between 1950 and 1955 BHC was the principal insecticide used against these species, but by the fall of 1955 this compound did not give satisfactory control in many areas of Florida. Gahan *et al.* (1956) found malathion, when tested as aerial sprays of fuel-oil solutions applied at dosages of ¼ to ½ pound per acre, to be effective against resistant adults of these two species. Since 1955 malathion has been the most widely used adulticide in Florida and today, 5 years after its first trial, is still producing excellent control. If these salt-marsh mosquitoes eventually do become resistant to this material, replacements will be urgently needed. Thus a continuous search is being conducted to find additional insecticides that can be used.

A laboratory testing program was initiated in 1957 to evaluate promising chemicals against adults of *A. taeniorhynchus*. This study is being conducted with selected chemicals that have produced at least 50 percent mortality of larvae of *Anopheles quadrimaculatus* Say at 1.0 p.p.m. The results of previous tests against *taeniorhynchus* adults have been reported by Davis and Gahan (1958) and Davis (1959). This paper presents the results obtained with the most effective of 53 additional compounds.

TESTING TECHNIQUE. Pupae from a laboratory colony were placed in small containers of water in groups of 1,000 to 2,000. The containers were placed inside cylindrical screen cages, 18 inches in length and 12 inches in diameter, closed on one end with plywood and on the other end with cloth sleeves (Fig. 1). Within

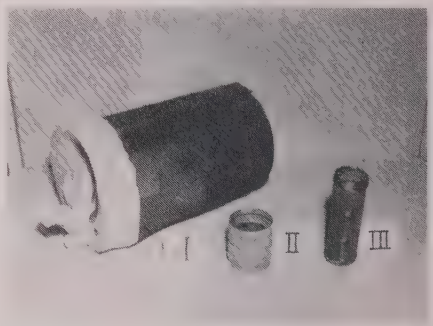

FIG. 1.—I, emergence cage; II, test cage; III, holding cage.

3 days after emergence the adults were immobilized in a room with a constant temperature of 36° F. and distributed into testing cages made of galvanized metal sleeves covered on each end with screen wire (Figs. 1, 2). Both males and females were used and each cage received about 25 adults. The mosquitoes were allowed to recover fully from the low temperature before being exposed to the insecticide.

The insecticides were dissolved in odorless kerosene and tested at concentrations ranging from 0.0025 percent to 0.25 percent in a wind tunnel, a cylindrical tube 4 inches in diameter through which a column of air was moved at 4 m.p.h. by a suction fan (Fig. 2). The cages of mosquitoes were placed in the center of the tube. One-fourth ml. of insecticidal solution was atomized at a pressure of 1 p.s.i. into the mouth of the tunnel, and the mosquitoes were exposed to the solution momentarily as it was drawn through the cage. Duplicate tests were run at each

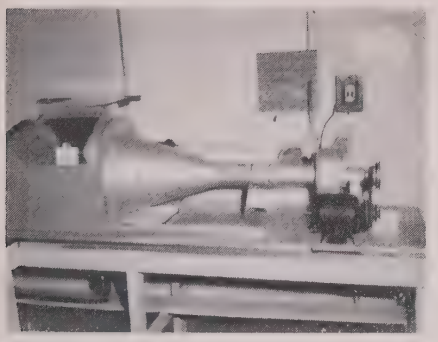

Fig. 2.—Wind tunnel. I, atomization chamber; II, funnel; III, test cage; IV, suction fan.

concentration. After treatment the mosquitoes were anesthetized with carbon dioxide, transferred to untreated screen holding cages (Figs. 1, 3), and held at a temperature of 84° F. and relative humidity of about 70 percent. A cotton pad saturated with a 20 percent honey-water solution was placed on the top of each cage and the mortality was recorded after 24 hours.

Effective Insecticides. The 15 insecticides listed below produced the highest mortalities and are considered sufficiently effective to merit testing in the field. The detailed results with these compounds and a malathion standard are given in Table 1.

Hercules AC-5727 (*m*-isopropylphenyl methylcarbamate)
Bayer 29952 (*O*-ethyl *O*-(*p*-methylthio) phenyl methylphosphonothioate)
Bayer 30554 (*O*-methyl *O*-(*p*-methylsulfinyl)phenyl methylphosphonothioate)
Dibrom (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate)
Bayer 30237 (*O*-methyl *O*-(*p*-methylthio)phenyl methylphosphonothioate)
Bayer 30468 (*O*-ethyl *O*-(*p*-ethylthio) phenyl methylphosphonothioate)
Dimethoate
Phosphamidon
American Cyanamid 12415 (*O,O*-diethyl *O*-[6-(3(2*H*)-pyridiazinonyl)] phosphorothioate)
DDVP
Union Carbide C&C-8305 (*p*-chloro-5-

TABLE 1.—Effectiveness of various insecticides in contact-spray tests against *Aedes taeniorhynchus* adults (6 tests)

Insecticide	Percent mortality after 24 hours at indicated concentration (percent)							LC-90		
	0.25	0.1	0.05	0.025	0.01	0.005	0.0025	Percent	Reciprocal ratio to malathion	Slope[a]
Hercules AC-5727	..	..	..	..	100	84	27	0.0057	5.75	4.6
Bayer 29952	..	..	..	100	91	50	..	.0103	3.18	3.5
Bayer 30554	..	..	100	99	94	49	..	.0108	3.04	3.3
Dibrom	..	..	..	100	83	17	2	.0133	2.47	4.7
Bayer 30237	..	100	99	97	87	51	10	.0162	2.02	2.7
Bayer 30468	100	98	99	96	67	27	..	.0251	1.31	2.2
Malathion	..	..	96	87	30	..	..	.0328	1.00	3.3
Dimethoate	100	99	97	86	51	12	..	.0338	.97	2.7
Phosphamidon	..	100	98	92	27	..	..	.0348	.94	3.8
American Cyanamid 12415	100	97	96	93	33	..	..	.0374	.88	2.3
DDVP	..	100	93	77	27	..	..	.0401	.82	3.2
Union Carbide C&C-8305	..	100	96	63	4	..	..	.0454	.72	4.3
Monsanto CP-13206	100	99	97	51	13	..	..	.0571	.57	2.8
Prolan	99	97	93	79	23	..	..	.0576	.57	2.1
Bulan	97	93	93	41	29	..	..	.0881	.37	1.9
Dilan	95	79	55	24	3	..	..	.0993	.33	2.6

[a] Slope of log dosage-probit mortality regression line.

methyl-*p*-thio-2,4,3-dioxaphosphabicyclo[4.4.0]decane)

Monsanto CP-13206 (*S,S'*-thiodimethylene bis(*O,O*-diethyl phosphorodithioate)

Prolan (1,1-bis(*p*-chlorophenyl)-2-nitropropane

Bulan (1,1-bis(*p*-chlorophenyl)-2-nitrobutane)

Dilan (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis (*p*-chlorophenyl)-2-nitrobutane)

Based on the computed LC-90 for each insecticide, six compounds were more toxic to adult mosquitoes than the malathion standard; Hercules AC-5727 was about 6 times as toxic, Bayer 29952 and Bayer 30554, 3 times, Dibrom and Bayer 30237, 2 times, and Bayer 30468, 1.3 times. The standard was about equal to dimethoate and phosphamidon, slightly better than American Cyanamid 12415 and DDVP, less than twice as effective as Union Carbide C&C-8305, Monsanto CP-13206, and Prolan, and more than twice as toxic as Bulan and Dilan.

Many materials that are highly effective against adult mosquitoes cannot be used in some situations because of their toxicity to mammals. For example, parathion, which must be used cautiously, has an acute oral LD-50 to rats of 3.5 mg./kg. (Lehman 1948), and DDT, which is considered relatively safe, has an acute oral LD-50 of 250 mg./kg. (Lehman 1950). Malathion has an acute oral LD-50 of 1,000 to 1,375 mg./kg. (Durham 1957), and is one of the safest insecticides known. Table 2 lists the acute oral toxicity to rats and some of the physical properties of the insecticides in Table 1 as given by their manufacturers. Based on this information Bayer 29952, Bayer 30554, and Bayer 30237 were more toxic than parathion and Bayer 30468 was only slightly less toxic. Phosphamidon and American Cyanamid 12415 also were highly toxic. DDVP, Hercules AC-5727, and Monsanto CP-13206 were 16, 4, and 2½ times, respectively, as safe as parathion. Dibrom was about twice as safe and Union Carbide C&C-8305 about ½ as safe as DDT, whereas dimethoate was about equal to DDT. The acute oral toxicities of Bulan and Dilan were about the same as reported for malathion.

SUMMARY. Wind-tunnel tests were con-

TABLE 2.—The physical properties of various insecticides and their toxicity to mammals [a]

Insecticide	Physical properties			Acute oral toxicity (LD-50) [b]
	State	Color	Odor	
Hercules AC-5727	Crystalline solid	White	Nearly odorless	15
Bayer 29952	Liquid	Yellowish	Very slight	1–2½
Bayer 30554	Liquid	Yellowish	Very slight	1
Dibrom	Liquid	Yellow	Slightly pungent	430
Bayer 30237	Liquid	Yellow	Rotton odor—strong	2½
Bayer 30468	Liquid	Yellowish	Sweet and weak odor	5–10
Malathion	Liquid	Amber	Pungent	1,000–1,375
Dimethoate	Solid	White	Pungent	185–325
Phosphamidon	Liquid	Off-white	Mild	[c]
American Cyanamid 12415	Crystalline solid	Yellow	Mild	Very toxic
DDVP	Liquid	Brownish	Nearly odorless	56–80
Union Carbide C&C-8305	Liquid	Clear amber	Mild	120
Monsanto CP-13206	Liquid	Amber	Slightly pungent	9
Prolan	Sticky semi-solid	Brown	Almond-like	[d]
Bulan	Sticky semi-solid	Brown	Almond-like	950
Dilan	Sticky semi-solid	Brown	Almond-like	1,100

[a] Based on information given by their manufacturer.
[b] Value for rats, based on mg./kg.
[c] LD-100, 50 mg./kg.
[d] No information available.

ducted with adults of *Aedes taeniorhynchus* (Wied.), to compare the toxicity of a malathion standard and a group of organic compounds known to be toxic to other species of insects. Among the chemicals superior to the standard were a carbamate and five organophosphorus compounds—Hercules AC-5727 (*m*-isopropylphenyl methylcarbamate), Bayer 29952 (*O*-ethyl *O*-(*p*-methylthio)phenyl methylphosphonothioate), Bayer 30554 (*O*-methyl *O*-(*p*-methylsulfinyl)phenyl methylphosphonothioate), Dibrom (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Bayer 30237 (*O*-methyl *O*-(*p*-methylthio)phenyl methylphosphonothioate), and Bayer 30468 (*O*-ethyl *O*-(*p*-ethylthio)phenyl methylphosphonothioate).

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CARBAMATE INSECTICIDES AS POTENTIAL MOSQUITO CONTROL AGENTS [1]

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The appearance of mosquito resistance to chlorinated hydrocarbons and more recently to organophosphorus insecticides makes it imperative that every new insecticide be thoroughly tested as a potential mosquito control agent.

Among the new types of insecticides, the carbamic acid esters have attracted much attention as a promising group of toxicants, especially since the introduction of Sevin® (1-naphthyl *N*-methylcarbamate) as an agricultural insecticide. A number of laboratories are now actively engaged in synthesizing carbamates for potential use as insecticides, and many such compounds have already passed the initial laboratory screening stage.

In the present work we have investigated the activity of a large number of carbamates in the laboratory against larvae and adults of the mosquitoes *Culex 5-fasciatus* and *Anopheles albimanus*. We present here the results obtained with the more toxic carbamates against these mosquitoes, and compare them with data on chlorinated hydrocarbon and organophosphorus insecticides.

Table 1 shows the LC_{50} values obtained with some typical chlorinated hydrocarbon, organophosphorus, and carbamate insecticides against *C. 5-fasciatus* and *A.*

[1] Paper presented at the Joint Meeting of the American Mosquito Control Association and the California Mosquito Control Association, Anaheim, California, January 30-February 2, 1961.

TABLE 1.—Comparison of toxicity of chlorinated hydrocarbon, organophosphorus, and carbamate insecticides against mosquito larvae

Compound	LC_{50} in p.p.m.	
	Culex 5-fasciatus (Susceptible)	*Anopheles albimanus* (Dieldrin resistant)
Dieldrin	0.0078	0.4
Lindane	0.025	0.21
DDT	0.07	0.015
Parathion	0.0032	0.0047
Baytex	0.0045	0.017
Malathion	0.08	0.12
N-methylcarbamate:		
m-sec-butylphenyl-[a]	0.03	0.13
m-isopropylphenyl-[b]	0.04	0.13
m-tert-butylphenyl-[c]	0.15	0.37
4-methylthio-3,5-xylyl-[d]	0.23	0.44
o-isopropoxyphenyl-[e]	0.35	0.24
4-dimethylamino-3,5-xylyl- (Zectran)[f]	0.49	1.4
o-isopropylphenyl-[g]	0.56	0.46
1-naphthyl- (Sevin)	1.0	0.62

[a] California Chemical RE5305.
[b] Union Carbide 10,856 and Hercules AC5727.
[c] California Chemical RE5030.
[d] Bayer 37344.
[e] Bayer 39007.
[f] Dow Chemical.
[g] Bayer 39731.

albimanus larvae. It is apparent that these carbamates are not very efficient larvicides, as compared to such compounds as dieldrin, lindane, DDT, parathion, and Baytex ® (*O,O*-dimethyl *O*-3-methyl-4-methylthiophenyl phosphorothionate).

There appears to be some discrepancy in the published information regarding the persistence of carbamates in water solution. David *et al.* (1960) reported that aqueous solutions of nine carbamates stored well for up to 14 days. Hassan & Hoskins (1960) also reported that the residual activity of *m*-isopropylphenyl *N*-methylcarbamate in water was maintained well over 30 days. On the contrary, Mulla & Axelrod (1960) found that the activity of this compound in water was lost within 24 hours. The latter data, however, were obtained at a temperature of 100° F., and this may account in part at least for the rapid hydrolysis of this compound.

Carbamates are known to be synergized by piperonyl butoxide and other methylene dioxyphenyl compounds against house flies (Moorefield, 1958). It was interesting, therefore, to see whether they could also be synergized against mosquitoes. The data in Table 2 show only limited synergism of carbamates by piperonyl butoxide against larvae of *C. 5-fasciatus*. The synergism ratios ranged from 1.5-fold for the more active compound to 7-fold for the least active one.

A more encouraging picture was obtained when various types of insecticides were compared on adult mosquitoes (Table 3). These tests were carried out by a contact method on filter paper in shell vials (Georghiou and Metcalf, 1961). The compounds are listed in order of decreasing efficiency against *C. 5-fasciatus*. Only two insecticides, DDVP, and lindane, were more toxic than the more active carbamates. DDVP and lindane are known to possess strong fumigant action and this may account, to some extent, for the high toxicity values obtained. There were five

TABLE 2.—Synergism of several carbamates with piperonyl butoxide (P.B.) against *Culex 5-fasciatus* larvae

Compound	LC_{50} in p.p.m. A (Alone)	B (1:5 P.B.)	Synergism ratio (A/B)
N-methylcarbamate:			
m-isopropylphenyl-	0.04	0.027	1.5
m-tert-butylphenyl-	0.15	0.045	3.3
4-methylthio-3,5-xylyl-	0.23	0.13	1.8
o-isopropoxyphenyl-	0.35	0.093	3.2
o-isopropylphenyl-	0.56	0.14	4.0
1-naphthyl- (Sevin)	1.0	0.22	4.5
N,N-dimethylcarbamate:			
1-isopropyl-3-methylpyrazolyl- (Isolan)	11.2	1.6	7.0

carbamates more toxic than Baytex and parathion, and nine carbamates more toxic than malathion. The carbamate *m*-isopropylphenyl *N*-methylcarbamate was outstanding with an LC_{50} of 0.2 μg./cm^2. Gahan *et al.* (1961) have shown that this compound has outstanding residual toxicity to several species of adult mosquitoes. However, in view of its mammalian toxicity (oral LD_{50} to the rat ca. 16 mg. per kg.) materials such as the *o*-isopropylphenyl- or *o*-isopropoxyphenyl *N*-methylcarbamates, which combine good activity against mosquito adults (Table 3) with oral LD_{50} values to the rat of about 300 and 200 mg. per kg. respectively, may be more suitable for use in human habitations.

Tests were also run on the effect of piperonyl butoxide on the toxicity of carbamates against adult mosquitoes. As in the case of larvae, the synergism ratios obtained with adults were low, especially in the case of the more active carbamates. There was a tendency for inverse relationship between the toxicity of the carbamate and the degree of synergism obtained. The most toxic compound (*m*-isopropylphenyl *N*-methylcarbamate) was potentiated the least, i.e., 1.3-fold, while the least toxic of those studied (Isolan ®) was potentiated 15 times. This relation was more evident in results with house flies (Metcalf *et al.* 1960) and appears to be correlated with the ability of the insect to detoxify the carbamate.

TABLE 3.—Comparison of toxicity of chlorinated hydrocarbon, organophosphorus, and carbamate insecticides against mosquito adults

Compound	LC_{50} in μg./cm^2 *Culex 5-fasciatus* (Susceptible)	*Anopheles albimanus* (Dieldrin resistant)
DDVP	0.088	0.094
Lindane	0.14	0.2
m-isopropylphenyl *N*-methylcarbamate	0.2	0.18
m-sec-butylphenyl *N*-methylcarbamate	0.43	0.4
m-tert-butylphenyl *N*-methylcarbamate	0.66	0.66
m-isopropylphenyl *N,N*-dimethylcarmabate	0.69	>10.0
Dieldrin	0.95	>100.0
o-isopropylphenyl *N*-methylcarbamate	1.02	0.32
Baytex	1.1	1.2
Parathion	1.2	0.46
carvacryl *N*-methylcarbamate	1.3	1.4
o-isopropoxyphenyl *N*-methylcarbamate	1.89	0.82
o-cyclopentyloxyphenyl *N*-methylcarbamate	3.3	3.1
m-tert-butylphenyl *N,N*-dimethylcarbamate	3.46	5.5
Malathion	5.9	4.4
DDT	6.9	0.23
4-methylthio-3,5-xylyl *N*-methylcarbamate	8.8	6.3

Of practical importance is the knockdown effect of carbamates. The results in Table 4 show that these compounds will

TABLE 4.—Comparison of speed of knockdown of chlorinated hydrocarbon, organophosphorus, and carbamate insecticides against *Culex 5-fasciatus* adults

Compound [1]	Time for 50% knockdown (Minutes)
DDT	50
Lindane	60
Dieldrin	253
Baytex	86
Parathion	116
N-methylcarbamate:	
m-tert-butylphenyl	41
4-dimethylamino-3,5-xylyl (Zectran)	43
m-isopropylphenyl	49

[1] Tested at LD_{95}.

produce a much quicker KD than will chlorinated hydrocarbon and organophosphorus insecticides. Quick KD in space sprays may be a disadvantage, since it may enable the insect to avoid accumulating a lethal dose of the insecticide. Unlike the carbamate KD on house flies, however, from which there is considerable recovery, our observations show that only a small fraction of the knocked-down mosquitoes will recover.

In summary, carbamates appear to be relatively ineffective against mosquito larvae. However, their high toxicity to adult mosquitoes, their pronounced knockdown effect, and the relatively low mammalian toxicity of some of them, make them a promising group of insecticides deserving further investigation.

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A COMPARISON IN TIME OF EGG HATCHING BETWEEN MALE AND FEMALE *AEDES AEGYPTI* (L.)

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Introduction. Recent observations in this laboratory have indicated a difference in the hatching rates of male and female larvae of *Aedes aegypti*. A review of the literature yielded little information although Rees (1901) stated that males of this species appeared to hatch faster than females.

Research was initiated to determine whether male and female larvae of *A. aegypti* hatched from eggs at differential rates. Additional factors studied were (1) the percentage of eggs that hatched during each time interval, (2) the subsequent percentage of eggs that hatched and the resulting sex ratio should the initial egg hatch be discarded at any period of time, and (3) a comparison of the mortality rates during rearing for larvae hatched at each time interval tested.

Materials and Methods. Eggs from the Fort Detrick strain of *A. aegypti* were used in the experiments and 18 replicates were conducted. The Rockefeller and Lagos strains were also used to check variation between strains.

For each replication five random lots of 20-day old eggs were inserted into separate pint jars containing distilled, de-ionized, autoclaved water. This water with its reduced oxygen tension served as the hatching stimulus. Each of the 5 lots of 5,000 eggs was submerged for a different test interval of 5, 10, 20, 40, and 60 minutes. Each lot of eggs was then removed and immediately submerged a second time in fresh autoclaved water until a total testing period of 60-minute duration was reached. This latter technique gave data on the number of eggs that hatched and the resulting sex ratios from discarding the initial hatch at different time intervals.

Samples of 440 newly emerged larvae from each hatching interval were hand-counted into 17 x 4½ x 2¼ inch white enamel trays. Each tray was filled and maintained with distilled, de-ionized water to a depth of 1.5 cm.

The larvae were fed ground dog pellets and yeast. The ground dog pellets were weighed and added at the rate of 0.2, 0.3, and 0.4 mg. per larva on days 0, 1, and 2 respectively. On days 3–9, 0.6 mg. of the ground dog pellets were added. The yeast, 0.001 ml. of a 22 percent suspension, was added on day 3.

The larvae were reared in constant climate rooms with an air temperature of 26.4° ± 0.8° C. and the relative humidity of 62.4 ± 5.1 percent. In this environment, the water in the trays remained at a temperature of 24.8° ± 0.4° C. When pupation began, the pupae were harvested and placed into pint ice cream cartons for emergence of the adult mosquitoes. Pupae were harvested until day 9 after hatching because only a few had not metamorphosed by this time.

Results and Discussion. There was a definite sex difference in the time required for larvae to hatch from the eggs as seen in Table 1. Male larvae hatched at a faster rate than females; this resulted in a predominance of males within the 0–10 minute interval. After this initial high percentage, the percentage of male larvae decreased and the percentage of female larvae increased with increasing time in the hatching fluid, a highly significant result statistically according to the following equation:

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$$Y = 48.42 - 26.39(.2^{x'})$$

Where:

Y = Percent females of the total adults and

$x = \frac{1}{20}$(Number of minutes in hatching fluid).

The percent of either sex of the total egg hatch was not linear as illustrated by the regression line of the female ratio in Figure 1. Figure 1 demonstrates that the percent of females increases only slightly after 45–50 minutes under the conditions of these experiments.

TABLE 1.—Effect of time and initial exposure to water of *Aedes aegypti* eggs, Fort Detrick strain, on percent of hatch, sex ratio, and rearing mortality

Time of eggs in water in minutes	% Hatch	% Male	% Female	% Mortality during rearing
0–5	15	66.0	34.0	8.5
0–10	34	64.7	35.3	8.7
0–20	54	56.7	43.3	8.4
0–40	91	52.1	47.9	9.4
0–60	96	52.9	47.1	7.9

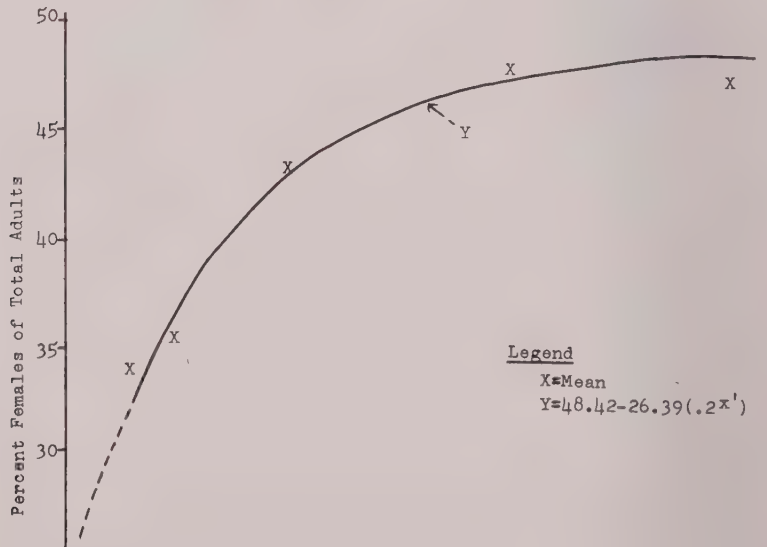

FIG. 1.—Regression of percent females of total adults on minutes in hatching fluid.

The sex pattern of hatching may be visualized as an overlapping of the distributions of males and females, as seen in Figure 2, with the total frequency of males being higher than the total frequency of the females. Although variation existed, this pattern was observed in each of the 18 replicates.

When the unhatched eggs were exposed to a second hatching stimulus, females were the predominant sex. This was not unexpected as many of the male larvae had previously hatched during the first submergence as was seen in Table 1. A summary of the replicates is contained in Table 2.

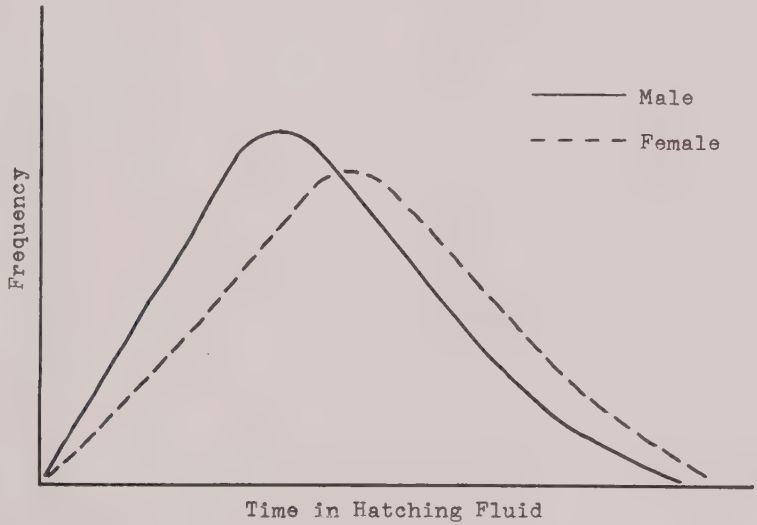

Fig. 2.—Hypothetical diagram demonstrating the sex pattern of hatching in *Aedes aegypti*, Ft. Detrick strain.

TABLE 2.—Effect of discarding initial egg hatch, time, and second exposure to water of *Aedes aegypti* eggs, Fort Detrick strain, on percent of hatch, sex ratio, and rearing mortality

Time of eggs in water in minutes	% Hatch	% Male	% Female	% Mortality during rearing
5–60	81	53.8	46.2	7.2
10–60	62	50.8	49.2	6.1
20–60	42	46.3	53.7	7.3
40–60	5	44.0	56.0	8.6

Only 4 percent of the eggs submerged for the entire 60 minutes failed to hatch. This percentage was also consistent for the eggs submerged for a shorter interval and resubmerged until a total period of 60 minutes was reached. The majority of the eggs were found to hatch between 10 and 40 mintues after initial exposure to the autoclaved water.

When the results obtained from the Fort Detrick strain were compared with the Rockefeller strain, little difference was observed in the hatching patterns. The percent males in the Rockefeller strain for 5, 10, 20, 40, and 60-minute hatching intervals was 66.0, 62.7, 58.3, 58.2, and 54.6, respectively. In the Lagos strain, the difference between the sexes in the

time of hatching was only observable during the first 5 minutes when 57.5 percent males hatched as compared with a 49.6 percent observed for the overall pattern. Although the number of larvae reared for the Lagos strain was only 880 for each time interval, this appears to be sufficient to indicate the existence of some variation between strains for this phenomenon.

The water used in hatching was tested in order to insure uniformity. The dissolved oxygen was 1.06 ± 0.52 ppm, the carbon dioxide was 0.61 ± 0.2 ppm, the temperature was $27.0 \pm 0.3°$ C., and the number of ions was less than 0.2 ppm.

Summary. In laboratory rearing of *Aedes aegypti*, eggs containing male larvae hatched somewhat faster than female. This early rate of hatching of larvae resulted in a predominance of males in the early hatch and females in the later. The results indicated the possibility of some strain variation for this phenomenon.

The majority of the eggs hatched between 10 and 40 minutes after being submerged in the de-oxygenated water. Ninety-six percent of eggs hatched when exposed for 60 minutes.

No significant mortality was observed in the larvae hatched during any specific time interval.

Acknowledgment

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INSECTICIDE-RESISTANT MOSQUITOES IN CALIFORNIA 1949–1961

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Although chemical control has been an integral part of mosquito abatement in California since the first districts were formed in 1915, resistance problems did not assume prominence until after the introduction of synthetic organic compounds in 1946. At this time DDT began to replace paris green, cresylic acid, the botanicals, and petroleum distillates.

DDT proved to be so effective as a mosquito larvicide that it appeared a panacea in chemical control had been achieved. The intense spray schedules carried out by abatement districts soon revealed, however, that DDT was not the ultimate in chemical control. In some cases only two seasons' control was obtained before resistance problems were encountered.

The first report of DDT resistance in California mosquitoes was by Gordon F. Smith (anonymous, 1949.) Despite several increases in dosage rate, the material was failing to control *Aedes* and *Culex* larvae in Kern County.

Bohart and Murray (1950) confirmed through laboratory tests that larvae of *Aedes nigromaculis* Ludlow had developed resistance to DDT in Tulare County.

Lindane, toxaphene, aldrin, and dieldrin were substituted in larviciding programs by districts that encountered difficulty in achieving control with DDT.

Gjullin and Peters (1952) reported on the degree and extent of resistance to DDT, lindane, toxaphene, and aldrin in several species of mosquitoes in the San Joaquin Valley. In general, it appeared that all chlorinated hydrocarbons were becoming ineffective for larviciding operations. The development of multiresistant strains precluded the further use of chlorinated hydrocarbons by many districts.

Even though mosquito larvae have developed rather extensive resistance to chlorinated hydrocarbons, some districts are still able to maintain control with these materials, particularly in the coastal areas.

Organophosphate insecticides have replaced chlorinated hydrocarbons where resistance problems have become severe. Gjullin, Isaak, and Smith (1953) demonstrated the effectiveness of EPN and other organophosphates in the control of chlorinated hydrocarbon resistant *A. nigromaculis* and *Culex tarsalis* Coq. Ultimately, parathion and malathion became the insecticides of choice in most mosquito abatement districts.

Malathion resistance was first detected in *C. tarsalis* collected in Fresno County by Gjullin and Isaak (1957). After two and one-half years of use, both larvae and adults exhibited considerable resistance to this compound.

Parathion failures against *A. nigromaculis* in the Kings Mosquito Abatement District near Hanford were confirmed in laboratory tests reported by Lewallen and Brawley (1958), Lewallen and Nicholson (1959). Parathion had been used for six years in the control program before resistance developed to a level sufficiently high to interfere with control. Even higher levels of parathion resistance have subsequently been found in *A. nigromaculis* larvae from Tulare County, with a resistance of the magnitude of 660 times being reported (Lewallen, 1961).

Detection of Resistance. When chemical control failures are noted in the field, physiological resistance is not always necessarily responsible. In treating water with a dense overgrowth of vegetation, spray applications sometimes fail to penetrate effectively, resulting in small amounts of toxicant reaching the larval source. Granular formulations are useful in overcoming this difficulty (Whitehead, 1951; Mulla, 1960; Rai and Lewallen, 1960).

Frequently pastures are irrigated in sections so that all stages of *A. nigromaculis* are present from one side of a field to the other. If the entire field is treated with a single application of insecticide, the more tolerant stages (eggs, pupae) survive, particularly with easily hydrolyzed organophosphates. Subsequent emergence of adults makes it appear that the material has failed to control the larvae to which it presumably was applied.

Inadequate coverage and sublethal concentrations can lead to control failures which may be interpreted by some observers as evidence of resistance.

Water with much organic matter, high alkalinity, mats of algae, dense vegetation, extremes in temperature and other factors may interfere with the action of toxicants.

Faulty materials account for some failures. Malathion, for example, must be stored in specially lined drums to prevent deterioration.

Generally, field evidence alone is not sufficient to substantiate the presence of resistance in mosquitoes since the variables mentioned above can result in misleading interpretations.

Cross-Resistance. DDT-resistance in *C. tarsalis* and *A. nigromaculis* does not induce immediate resistance to lindane, toxaphene, aldrin or dieldrin, but resistance to these compounds appears to develop

more rapidly if DDT-resistance has occurred first. Some species of mosquitoes exposed only to dieldrin with subsequent development of dieldrin-resistance, remain susceptible to DDT (Brown, 1958).

Most of the work done on characterizing chlorinated hydrocarbon resistance has been done on the house fly. Chlorinated hydrocarbon resistance in the house fly is manifested in two specific types that are genetically distinct (Busvine, 1954; Metcalf, 1955; Kearns, 1955). One type confers resistance to DDT and its analogs; the other type confers resistance to lindane, dieldrin and other cyclodiene compounds. This separation into two distinct types also applies to mosquitoes (Brown, 1960).

Resistance to chlorinated hydrocarbons does not confer cross-resistance to organophosphorous compounds. House flies that develop a high level of organophosphorous resistance also acquire a cross-resistance to chlorinated hydrocarbons. This phenomenon appears to happen even when there has been no previous history of treatment with chlorinated hydrocarbons (Brown, 1960).

This relationship does not necessarily hold for mosquitoes, as evidenced by findings on the malathion-resistant strain of *C. tarsalis* from Fresno. Malathion was used against this strain because high levels of chlorinated hydrocarbon resistance had developed. Despite pressure from malathion applications, the chlorinated hydrocarbon resistance decreased (Lewallen, 1960).

When resistance has developed to a given organophosphate, cross-resistance to other organophosphates is not necessarily conferred. The malathion-resistant strain of *C. tarsalis* remains susceptible to parathion and other organophosphates (Gjullin and Isaak, 1957). Parathion-resistant *A. nigromaculis* remain susceptible to methyl parathion and other organophosphates (Lewallen and Nicholson, 1959).

Natural Resistance vs. Acquired Resistance. In assessing the value of a toxicant for the control of mosquito larvae, it is advantageous to obtain information on more than a single species since the response to synthetic organic compounds varies considerably with different species. A case in point concerned the promotion and development of DDVP as a mosquito larvicide based on information obtained from *Culex* sp. only (Isaak, 1957; McFarland, 1957). Although the dosage recommended for the control of mosquito larvae was adequate for *Culex* sp. it was found that several times the recommended dosage was required for control of *A. nigromaculis* (Lewallen, 1958b). The material proved to be too expensive for economical application at the dosage required. This expression of natural resistance is distinct from acquired resistance where the toxicant works well at first, becoming ineffective after continued usage.

Determination of Resistance Levels by Laboratory Tests. In order to establish the degree of resistance in a strain, a base line for comparison must be established. Usually this is done by obtaining information on the response of a susceptible strain and comparing the results with those obtained on the suspect resistant strain. The magnitude of resistance will depend on the susceptibility level of the strain used for comparison.

Considerable variation in susceptible strains has been noted. The susceptible strain of *C. tarsalis* maintained at the United States Department of Agriculture laboratory at Corvallis, Oregon, is about ten times more susceptible to DDT than the susceptible strain maintained at the Bureau of Vector Control laboratory at Fresno. The LC_{50} and LC_{90} for DDT in the Corvallis strain is 0.003 ppm and 0.01 ppm, respectively (Eddy *et al.*, 1958). At Fresno the LC_{50} is 0.03 ppm and the LC_{90} equals 0.129 ppm.

In establishing base lines for resistance studies of *A. nigromaculis* it has been necessary to field-collect susceptible strains, since this species has not been colonized. Mosquito abatement and agricultural pest control have become so intense in California that it is difficult to collect material in areas free from insecticide influence. As an illustration, the Pinedale strain of *A. nigromaculis* used as a base line in

1958 for parathion-resistance studies (Lewallen and Brawley, 1958; Lewallen and Nicholson, 1959) has since that time been under treatment with parathion for two seasons. No data were obtained on this strain in 1960 but parathion susceptibility tests were made in 1958, 1959, and 1961. Results of the three years' tests are shown in Table 1 which shows a four-fold LC_{50}

TABLE 1.—Susceptibility levels to parathion of *Aedes nigromaculis*, Pinedale, 4th instar

Year	LC_{50} in ppm	LC_{90} in ppm
1958	0.000035	0.0001
1959	0.000045	0.00013
1960	..	..
1961	0.00013	0.0008

and eight-fold LC_{90} decrease in susceptibility level between 1959 and 1961.

Substitute Insecticides for Resistant Strains. The substitution of organophosphates to control chlorinated hydrocarbon-resistant mosquitoes has been complicated by subsequent resistance to the substitute compounds (Gjullin and Isaak, 1957; Lewallen and Nicholson, 1959). Although it appears that continued substitution of other organophosphates will be successful in regaining control, the question remains as to how long the substitutes will remain effective. Methyl parathion has given satisfactory control of parathion-resistant *A. nigromaculis* for two seasons (Lewallen, 1960).

In developing new insecticides the trend has been toward organophosphates; consequently, a large variety of compounds of this type is available to select from in a substitute insecticide program. Relying so heavily on one type of insecticide may impose serious limitations eventually upon the success of substitutes in chemical control programs. For this reason increased attention has been focused recently on the development of other types of compounds. Carbamate insecticides have been tested against mosquito larvae in California with promising results (Mulla *et al.*, 1960). The ease with which resistance develops to this type of compound in house flies, however, may be indicative of its limitations in mosquito control (Georghiou *et al.*, 1961; Georghiou and Metcalf, 1961).

Chrysanthemumic acid esters have also been tested against various species and strains of mosquitoes in California (Gjullin and Lewallen, 1958; Lewallen and Gjullin, 1960). Economic considerations based on necessary high rates of application have restricted the use of this type of material for mosquito control.

Research on New Compounds for Resistant Strains. Most of the work on compounds designed specifically against resistant strains has been directed toward studies on the house fly. Cetyl bromoacetate has been reported to be more effective against DDT-resistant house flies than against a susceptible strain (Ascher, 1957, and 1958).

Bold, new approaches such as the search for a negatively correlated insecticide as cited above, the use of chemosterilants (LaBrecque *et al.*, 1960), and application of mitotic poisons (Mitlin *et al.*, 1957) must be pursued if we are to keep ahead of the development of resistant strains. Another possibility that offers promise in the attempt to develop new insecticides for mosquito and fly control may be found in the synthesis of materials similar in nature and action to naturally occurring insect hormones. The feasibility of this approach has been pointed out previously (Knipling, 1958; Lewallen, 1958). Work on naturally occurring hormones has progressed to the point where structures are near the point of elucidation (Karlson, 1959). Naturally occurring hormones have already been demonstrated as having insecticidal potential (Williams, 1956; Gilbert and Schneiderman, 1958).

Based solely on empirical formulae, attempts have been made to synthesize molecules similar in structure to the molting hormone in our laboratory. The literature has indicated that some of the insect hormones may be steroids (Gilbert and Schneiderman, 1958). Oddly enough, one of the materials synthesized that had a fair degree of insecticidal activity was an aliphatic ester. Since the basis for forming

this compound was derived solely from empirical considerations, it is not known whether the activity observed was hormonal or not. Tests were performed on mosquito larvae, and a fairly high degree of mortality was observed. Even if the compound does not approximate a naturally occurring growth regulator, it represents an entirely new type of compound that exhibits insecticidal properties that may be improved by a study of synthesized analogs. Details of the synthesis and experimental results will be published elsewhere.

Although there have been numerous setbacks in the chemical control of mosquitoes due to the development of resistance, by and large it has been possible to cope with the problem through substitute insecticides. With the threat of development of resistance to materials now in use against mosquitoes, as well as other arthropods of public health importance, research agencies are hard at work to extend the present array of insecticides. Only through sustained, penetrating research on this problem can the challenge offered by the perplexing problem of resistance be met, and the high standards of mosquito control technology be maintained.

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STUDIES ON THE INHERITANCE OF RESISTANCE TO DDT AND TO MALATHION IN THE MOSQUITO, *CULEX TARSALIS* COQ.

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A colony of *Culex tarsalis* Coq. highly resistant to DDT was collected from a log pond at Oak Ridge, Oregon, in 1956 and has been maintained at the Corvallis laboratory. Data on the amount of resistance to DDT present in the colony and on rearing techniques have been reported (Eddy *et al.* 1958).

A malathion-resistant strain of *tarsalis*, collected near Fresno, Calif., in 1957 is also being reared in the laboratory. The colony was about 100 times resistant to malathion at the time these experiments were undertaken and also about 2 times resistant to DDT. Initially it was about 75 to 80 times resistant to malathion and 5 times to DDT. The extent of cross-resistance to other insecticides present in this colony has been reported (Darrow and Plapp 1960).

In the present work the inheritance of insecticide resistance in these colonies was studied. Data are also presented on levels of cholinesterase (ChE) and aliphatic esterase (Ali-E) activity in the same colonies. Measurements of enzyme levels in resistant strains of insects have been of special interest since the finding that low levels of aliesterase are related to organophosphate resistance in the house fly (*Musca domestica* L.) (Asperen and Oppenoorth 1959, Bigley and Plapp 1960).

MATERIALS AND METHODS. Insects.—The DDT-resistant colony from Oak Ridge and the malathion-resistant colony from Fresno were the two resistant colonies used in these experiments. The insecticide-susceptible colony was obtained from the Rocky Mountain Laboratory, Hamilton, Mont., and has been reared for several years in the laboratory without exposure to insecticides. For the sake of brevity, the three colonies will be referred to as DDT-R, Mal-R, and Reg, respectively.

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The three colonies were maintained in the laboratory in separate rearing rooms. The methods employed in caring for them have been previously described (Eddy *et al.* 1958).

At the time of these experiments, levels of insecticide resistance in the DDT-R and Mal-R colonies had been maintained with little change for at least a year. In the Mal-R colony resistance was maintained by exposing fourth-instar larvae to 2 p.p.m. of malathion for 24 hours every third or fourth generation. Resistance in the DDT-R colony was maintained by pressuring fourth-instar larvae with 1.5 p.p.m. of DDT every second generation.

Genetic Technique.—Virgin females from the different colonies were obtained as newly emerged adults. They were collected within 24 hours of emergence. Previous work in the laboratory had shown that no mating took place until the females were at least 36 hours old (Darrow, unpublished data). Young males were obtained from the colonies at the same time the females were collected. Populations of 100 females were placed in approximately 1-cubic-foot cages with an equal number of males from one of the other colonies. All six possible crosses between the colonies were made. When the adults were 4 days old, the females were fed a blood meal by placing a young white mouse in the cage. Four days later a pan of water was placed in the cage. Maximum egg production occurred within 48 hours. Adults were fed sugar water at all times.

Larvae from the different crosses were reared routinely. Pupae of the F_1 generation were placed in cages and the emerging adults were allowed to mate to produce the F_2 generation. Several backcrosses were made in which females from the F_1 generation were mated with males from the parent colony which possessed the opposite character from the F_1 in regard to insecticide resistance.

Measurement of Resistance.—The susceptibility to DDT and to malathion of the parent colonies and of progeny from the various crosses was determined in tests with fourth-instar larvae. As far as possible 7-day-old larvae were used so that the tests could be completed before pupation took place, usually about 10 days after hatching. The two insecticides were tested with larvae at 6 concentrations, 0.01, 0.03, 0.1, 0.3, 1, and 3 p.p.m. These concentrations were sufficient to cover the complete range of susceptibility from the LC-50 with the most susceptible strain to nearly an LC-50 with the most highly resistant strain. The tests were conducted in 1-pint glass jars containing 250 ml. of water to which 20 larvae were added. The insecticides were then pipetted into the jars in either 0.3 or 1 ml. of acetone. Larvae were held at a temperature of about 25° C. Mortality determinations were made 24 and 48 hours later.

Determinations of Esterase Activity.—Measurements of ChE activity and of Ali-E activity were made by the colorimetric method of Hestrin (1949). Acetylcholine bromide at 2.5×10^{-3} M was the substrate employed for ChE measurements. Tributyrin at 1×10^{-3} M was used rather than methyl *n*-butyrate, the substrate employed in determining Ali-E activity in the house fly, because we found that esterase activity against methyl *n*-butyrate is not present in measurable amounts in *tarsalis*. Homogenates of larvae of the several colonies and crosses were prepared at a concentration of 10 insects per ml. One ml. of the homogenate, a ml. of pH 7.2 phosphate buffer, and a ml. of substrate were incubated at 37° C. for 15 minutes for esterase activity determinations. Details of the method have been described previously (Bigley and Plapp 1960).

RESULTS. The measurements of susceptibility to malathion are summarized in Table 1. The data show that the DDT-R and Reg colonies were equally susceptible to malathion with LC-50's of between 0.03 and 0.1 p.p.m. With the Mal-R colony, the LC-50 was slightly above 3 p.p.m. Mortalities obtained with F_1 larvae in the crosses in which a Mal-R parent was used

TABLE 1.—Mortalities of *Culex tarsalis* larvae after 24 hours of exposure to malathion in distilled water

Colony or cross	Generation	Mortality at indicated p.p.m. (%)					
		0.01	0.03	0.1	0.3	1.0	3.0
Regular	Parent	0	20	100	100	100	100
Malathion-R	"	0	0	0	0	0	26
DDT-R	"	4	22	100	100	100	100
Reg ♀ x Mal-R ♂	F_1	0	0	0	0	0	100
"	F_2	0	26	18	22	38	90
Reg ♀ x DDT-R ♂	F_1	6	62	100	100	100	100
"	F_2	0	88	100	100	100	100
Mal-R ♀ x Reg ♂	F_1	0	0	0	0	46	100
"	F_2	0	22	32	30	24	94
Mal-R ♀ x DDT-R ♂	F_1	0	0	0	2	60	100
"	F_2	0	24	20	26	20	92
DDT-R ♀ x Reg ♂	F_1	2	30	92	100	100	100
"	F_2	0	78	100	100	100	100
DDT-R ♀ x Mal-R ♂	F_1	0	0	0	0	64	100
"	F_2	2	12	14	24	34	94
(Mal-R ♀ x Reg ♂) ♀ x Reg ♂	Backcross	0	6	22	30	34	98

show that resistance is dominant over susceptibility. No evidence was obtained for sex-linkage since the F_1 larvae were resistant to malathion whether Mal-R males or females were used. However, the mortalities obtained at 1 and 3 p.p.m. in the F_1 were higher than those for the resistant parent colony, which indicated that resistance was not completely dominant.

The experiments with the F_2 generation were in agreement with a single gene hypothesis for inheritance of resistance. The expected 3:1, resistant:susceptible, ratios were obtained for all four crosses involving a Mal-R parent. Mortalities with F_2 larvae were lower than with F_1 larvae at 3 p.p.m. (92.5 percent average vs. 100 percent); thus further evidence was provided of incomplete dominance for the gene which conferred resistance.

However, in the backcross experiment, in which resistant F_1 females were mated with susceptible colony males, the expected 1:1 ratio was not obtained, but rather the same 3:1 ratio as in the F_2 generation. The same results were obtained when the experiment was repeated several months later. We do not know the reasons for the failure to obtain the expected ratio. It is possible that additive gene action or some modifiers of the major gene for resistance are involved.

The measurements of susceptibility to DDT are summarized in Table 2. The data show that some resistance to DDT was also present in the colony selected with malathion, since the LC-50 was between 0.03 and 0.1 p.p.m., whereas the figures were about 0.01 and 1 p.p.m. for the Reg and DDT-R colonies, respectively.

The bioassays of the F_1 generation showed that susceptibility to DDT was dominant over resistance. In all six crosses the mortalities obtained with F_1 larvae were nearly identical with those of the more susceptible of the two parents. That resistance was recessive to susceptibility was demonstrated in the F_2 generation where all tests showed that the larvae were less susceptible than their F_1 parents. The results of the backcross experiment were in agreement with those expected because the larvae were far less susceptible to DDT than were their F_1 parents.

From the data we concluded that in the strains tested, DDT resistance is recessive to susceptibility. The results are comparable to those discussed by Davidson & Jackson (1961) who showed that DDT resistance in mosquitoes is usually reces-

TABLE 2.—Mortalities of *Culex tarsalis* larvae after 24 hours of exposure to DDT in distilled water

Colony or cross	Generation	Mortality at indicated p.p.m. (%)					
		0.01	0.03	0.1	0.3	1.0	3.0
Regular	Parent	56	78	100	100	100	100
DDT-R	"	4	8	14	28	60	54
Malathion-R	"	8	12	76	92	100	100
Reg ♀ x DDT-R ♂	F_1	44	76	100	100	100	100
"	F_2	8	26	64	76	84	98
Reg ♀ x Mal-R ♂	F_1	26	90	98	100	100	100
"	F_2	10	84	94	100	100	100
DDT-R ♀ x Reg ♂	F_1	56	90	94	98	100	100
"	F_2	38	62	96	88	96	100
DDT-R ♀ x Mal-R ♂	F_1	30	24	78	98	100	100
"	F_2	14	30	62	84	96	100
Mal-R ♀ x Reg ♂	F_1	4	84	100	100	100	100
"	F_2	28	74	100	100	100	100
Mal-R ♀ x DDT-R ♂	F_1	4	2	90	100	100	100
"	F_2	24	22	64	94	84	98
(DDT-R ♀ x Reg ♂) ♀ x DDT-R ♂	Backcross	12	30	60	68	86	94

sive to susceptibility. The inheritance appears to be monogenetic although it is difficult to make the statement with finality from the data presented. In the present study the change in response in the DDT-R colony has been shown to be not so much a shift in the location of the dosage-mortality curve as a shift to a different type of response with a dosage-mortality curve having a lower slope. It is only on this basis that we can explain the fact that after several years of pressuring, individuals highly susceptible to DDT are still present in the DDT-R colony.

Of further interest is the fact that DDT resistance in the Mal-R strain is inherited in a different manner from malathion resistance in the same strain. This finding may be contrasted to results obtained when house flies have been pressured with organophosphates. Selection with organophosphates often induces a very high level of cross-resistance to DDT (March 1959). As demonstrated, no genetic relationship exists between the two types of resistance present in the *tarsalis* colony selected with malathion.

The measurements of ChE and Ali-E activity in the different colonies are summarized in Table 3. Levels of ChE were similar in Reg and Mal-R larvae. Surprisingly, ChE activity in the DDT-R colony was about twice as great as in the other colonies. Measurements of the F_1 progenies showed that inheritance of high levels of ChE was recessive to inheritance of low levels. Although inheritance of ChE levels is similar to the pattern of inheritance of DDT resistance, far more detailed studies are needed to demonstrate a causal relationship, and for the present, the similarity is merely noted.

TABLE 3.—Cholinesterase and ali-esterase activity in *Culex tarsalis* larvae

Colony or cross	Micromoles of substrate hydrolyzed per 15 minutes per 10 mosquito larvae	
	Acetylcholine (Che)	Tri *n*-butyrin (Ali-E)
Regular	0.725	1.68
Malathion-Resistant	0.65	1.88
DDT-Resistant	1.425	1.84
Reg ♀ x Mal-R ♂	0.50	1.68
Reg ♀ x DDT-R ♂	0.925	2.08
Mal-R ♀ x Reg ♂	0.65	1.68
Mal-R ♀ x DDT-R ♂	0.80	1.64
DDT-R ♀ x Reg ♂	0.675	1.64
DDT-R ♀ x Mal-R ♂	0.60	1.52

Ali-E activity as determined with the substrate tributyrin was similar in all colonies, and lowest in the Reg colony. Thus, unlike organophosphate-resistant house flies, inheritance of low levels of activity toward a substrate containing butyrate esters does not appear to be related to malathion resistance. Further evidence that the mechanism of resistance to malathion in *tarsalis* differs from that in the house fly has been indicated previously (Darrow and Plapp 1960). In that study it was shown that the pattern of cross-resistance to other insecticides in the Mal-R colony differed significantly from the typical pattern occurring in the house fly.

Summary. 1. Experiments have demonstrated that resistance to malathion in the Fresno strain of *Culex tarsalis* is dominant over susceptibility.

2. DDT resistance, present at a high level in the Oak Ridge strain of *tarsalis* and at low levels in the Fresno strain, is recessive to susceptibility.

3. Cross-resistance to DDT in the strain selected with malathion appears to be caused by a separate factor and is not a result of selection with malathion as often happens with the house fly.

4. Quantitative changes in levels of either cholinesterase or ali-esterase are not related to malathion resistance, but high cholinesterase levels present in the Oak Ridge strain are inherited in the same manner as is resistance to DDT.

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SUSCEPTIBILITY OF VARIOUS LARVAL INSTARS OF *CULEX P. QUINQUEFASCIATUS* SAY TO INSECTICIDES [1]

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Field and laboratory larviciding tests against mosquitoes are generally directed against the fourth instar larvae (Isaak 1956, Lewallen 1958, Mulla 1961, Mulla *et al.* 1960, 1961). The fourth instar larvae are considered by many workers to be more tolerant to larvicides than the younger instar larvae and, therefore, practically all larvicidal evaluation studies have been conducted on the response of the fourth instar larvae to candidate insecticides. Materials that would cause fourth instar larval mortality at a specific dosage are generally believed to produce a similar if not a greater response in the younger instar larvae.

The common belief that fourth instar larvae are more tolerant to organophosphorus and other insecticides than the younger larvae, has not been supported by experimental evidence. Information on the susceptibility level of various instar larvae to the newer insecticides is lacking. From the standpoint of screening programs and field evaluation studies, information on the susceptibility level of various instar larvae to insecticides could be of some value.

In laboratory screening tests it is often difficult to obtain a pure stock of fourth instar larvae for establishing dosage response lines of test materials. A number of third instar larvae are usually mixed with the fourth instar larvae; separation of these is time-consuming and often impractical. The accuracy and reproducibility of the dosage response lines may be influenced among other things by the number of third instar larvae present, as well as by the degree of their susceptibility to insecticides. If the susceptibility level of the third instar larvae to insecticides approximates that of the fourth instar larvae, the variation introduced due to the presence of third instar larvae may be very small; on the other hand, if the susceptibility level of the third instar larvae is appreciably greater than that of the fourth instar larvae, the variation introduced by heterogeneous populations may be of greater magnitude. Variation of this nature is also encountered in field evaluation work where, at times, the third instar larvae are not easily differentiated from the early fourth instar larval population.

In field mosquito larvicidal treatments it is desirable to know the susceptibility level of various larval instars to the larvicidal materials. After application, the larvicides disappear due to physical and chemical processes, and the administered concentration of the larvicide decreases. Some larvicides degrade faster than others. The initial dosage applied for the control of fourth instar larvae also affects younger instar larval populations present at the time of treatment. In most cases the treated breeding source is subject to repeated reinfestation; first instar larvae may appear within a few days after treatment. If these are highly susceptible to the larvicidal material employed, complete kill may be obtained, despite the fact that the concentration of the toxicant in the water was diminished markedly. Stability of larvicidal materials and the level of susceptibility of the first instar larvae to these will determine the extent of control following a specific application.

To obtain information on the susceptibility level of various larval instars, the current studies were initiated in the laboratory against first, second, third and

[1] Paper No. 1326, University of California Citrus Experiment Station, Riverside, California.
[2] The able assistance of H. Axelrod of the Department of Entomology, University of California, Riverside, is duly acknowledged.

fourth instar larvae of the mosquito *Culex pipiens quinquefasciatus* Say.

Methods and Materials. Larvae from a susceptible colony of *Culex p. quinquefasciatus* Say were used. The colony had been reared for several years without being exposed to selection pressure from insecticides. First instar larvae were obtained by placing egg rafts in tap water. Soon after hatching, the larvae were transferred to tap water in cups by means of a fine plastic screen scoop. Second and third and fourth instar larvae were removed from colony trays and transferred to the cups in a similar manner.

The materials were evaluated in the same manner as described elsewhere (Mulla *et al.* 1960, 1961). Twenty-five larvae of each instar were added to 100 ml. of tap water in 6-ounce paper cups. Acetone solutions of the toxicants were added to the cups after addition of the larvae.

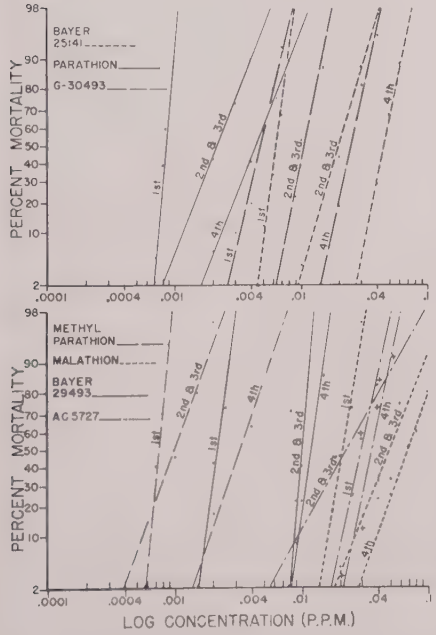

Fig. 1.—Dosage response lines of insecticides against various larval instars of *Culex p. quinquefasciatus* Say.

Larval mortality was recorded after an exposure period of 24 hours at a temperature of 78–80° F. Each cup was replicated twice and each material was run 2 or 3 times on different days.

The average 24-hour mortality of the larvae for each concentration of each material was plotted on a probit log paper. The points were visually joined by a straight-line. LC_{50} and LC_{90} levels were read off these dosage response lines.

Results and Discussion. Eleven insecticides were evaluated against the various larval instars of *Culex p. quinquefasciatus* Say. Of these insecticides one was an organochlorine, two were carbamates and the others were organophosphate insecticides. The dosage response lines of these eleven materials against the first, second and third and fourth instar larvae are presented in Figures 1 and 2.

The first instar larvae were highly susceptible to parathion. The dosage response line of this material against the newly hatched larvae was steep (Fig. 1). The dosage response lines of parathion against the second and third, and fourth stage larvae were not as steep as the line against the first stage. The dosage response lines of G-30493 [*O,O*-dimethyl *S*(-3,4-dichlorophenylthio) methyl phosphorodithioate] against the various larval instars indicated similar slopes. Bayer 25141 [*O,O*-diethyl *O*-*p*-(methylsulfinylphenyl) phosphorothioate] showed high activity against the first instar larvae with a relatively steep dosage response line. Dosage response lines of Bayer 25141 against the second and third, and fourth instar larvae became flatter.

Methyl parathion, like parathion, manifested a high degree of activity against first instar larvae with a steep dosage response line (Fig. 1). The dosage response lines of methyl parathion against second and third, and fourth instar larvae were flatter and showed approximately the same slope. Bayer 29493 [*O,O*-dimethyl *O*-[4-(methylthio)-*m*-*tolyl*] phosphorothioate] proved highly effective against the first instar larvae but showed decreased activity against second and third, and fourth instar

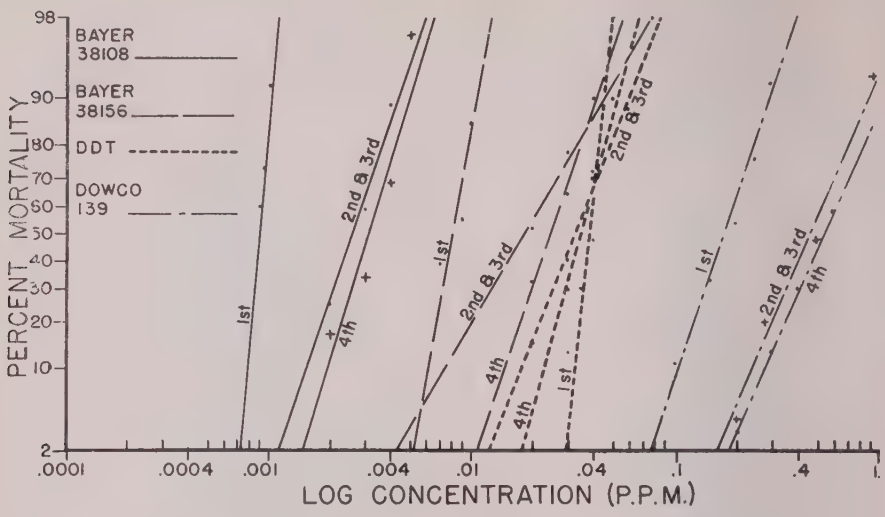

FIG. 2.—Dosage response lines of insecticides against various larval instars of *Culex p. quinquefasciatus* Say.

larvae. Essentially no difference in activity of Bayer 29493 was determined against second and third, and fourth instar larvae. The degree of activity of AC-5727 (*m*-isopropylphenyl *N*-methylcarbamate) was essentially the same against all larval instars. Malathion showed more activity against the first instar larvae but its spectrum of activity against the second and third, and fourth instar larvae was, for all practical purposes, the same.

Bayer 38108, like parathion and methyl parathion, showed higher activity against the newly hatched larvae (Fig. 2). How-

TABLE 1.—Susceptibility of various instar larvae of *Culex p. quinquefasciatus* Say

Material	LC_{50} (PPM)			LC_{90} (PPM)			Susceptibility Fold LC_{50}[a]		Susceptibility Fold LC_{90}[a]	
	First	Second and third	Fourth	First	Second and third	Fourth	First	Second and third	First	Second and third
Methyl parathion	.00074	.0010	.0032	.00086	.0017	.0055	4.3	3.2	6.4	3.2
Parathion	.00085	.0022	.0043	.00096	.0040	.0081	5.0	1.9	8.4	2.0
Bayer 38108	.00089	.0025	.0030	.0010	.0042	.0048	3.4	1.2	4.8	1.1
Bayer 29493	.0021	.010	.012	.0026	.0115	.0150	5.7	1.2	5.8	1.3
G-30493	.0047	.010	.030	.0068	.0145	.034	6.4	3.0	5.0	2.4
Bayer 25141	.006	.020	.045	.0075	.032	.062	7.5	2.2	8.3	1.9
Bayer 38156	.008	.019	.025	.010	.046	.042	3.1	1.3	4.2	0.9
Malathion	.020	.041	.069	.027	.11	.12	3.5	1.7	4.5	1.1
AC-5727	.030	.024	.036	.042	.058	.050	1.2	1.5	1.2	0.9
DDT	.038	.032	.035	.046	.060	.053	0.9	1.1	1.2	0.9
Dowco 139	.17	.48	.56	.29	.93	1.15	3.3	1.2	4.0	1.2

[a] Compared to fourth instar larvae.

ever, it manifested no marked differential activity against the second and third and fourth instar larvae. Similarly, Bayer 38156 (*O*-ethyl *S*-*p*-methylphenyl ethylphosphorodithioate) showed high activity against first instar larvae. Its activity against the second and third and fourth instar larvae, was lower but practically the same against these instars. DDT indicated a similar range of activity against all larval instars. Dowco 139 or Zectran (4-dimethylamino-3,5-xylol-*N*-methylcarbamate) showed higher activity against the newly hatched larvae, but the range of activity was lower and essentially the same against the other larval instars.

The degree of susceptibility to insecticides of first, second and third and fourth instar larvae may be easily visualized in terms of their LC_{50} and LC_{90} concentrations (Table 1). An analysis of the degree of susceptibility to toxicants of first, second and third instar larvae, as compared to the susceptibility of the fourth instar, indicates some interesting trends. It is obvious from Table 1 that the various larval instars do not show a uniform degree of susceptibility to all the toxicants used.

The first instar larvae were found more susceptible than the fourth instar larvae (both at LC_{50} and LC_{90} levels) to all the insecticides tested except AC-5727 and DDT. The first and fourth instar larvae were equally susceptible to AC-5727 and DDT.

The second and third instar larvae were found to be more susceptible (both at LC_{50} and LC_{90} levels) to methyl parathion, parathion, G-30493, and Bayer 25141 than the fourth instar larvae. The degree of susceptibility of second and third and fourth instar larvae to Bayer 38108, Bayer 29493, Bayer 38156, malathion, AC-5727, DDT and Dowco 139 (Zectran), within the range of experimental variation, was essentially the same.

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The Eighth Annual Conference of the **ILLINOIS MOSQUITO CONTROL ASSOCIATION** will be held on March 30–31, 1962, at the Dixie Governor Motel, East Hazelcrest, Illinois. The program will include scientific papers, descriptions of adequate mosquito abatement techniques, demonstration of equipment and other features. The location is readily accessible to all individuals who travel by automobile from Indiana, Michigan, Wisconsin, and Ohio. Details concerning the meeting can be obtained from Harvey J. Dominick, Secretary-Treasurer, Illinois Dept. of Public Health, Div. of Sanitary Engineering, Springfield, Illinois or from Dr. Robert Hedeen, Manager, South Cook County Mosquito Abatement District, P. O. Box 30, Harvey, Illinois.

ABANDONED MINES AS OVERWINTERING SITES FOR MOSQUITOES, ESPECIALLY *CULEX TARSALIS* COQ., IN NEVADA [1]

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INTRODUCTION. Since *Culex tarsalis* is considered the principal vector of western encephalitis (WE) in the West, much emphasis has been placed on discovering the sites of overwintering females. Hibernation of females in loose rock at bases of volcanic outcrop and in manmade rock piles in Washington was reported by Rush *et al.* (1958). Loomis and Green (1955) reported females of this species throughout the winter in rodent burrows in the San Joaquin Valley of California, whereas Keener (1952) observed them overwintering in Nebraska in food-storage cellars. Price *et al.* (1960) reported on a 3-year survey in Minnesota of many sites, including 27 caves, and found only a few present and none after November 27. In Colorado, Blackmore and Winn (1956) found many females present in abandoned mines, but they did not clearly state whether these populations overwintered successfully. Dow *et al.* (1956) observed small numbers of *tarsalis* in two abandoned mines in northern Utah but concluded that the mines were probably not suitable as hibernation quarters.

This study was initiated since some observations in abandoned mines in the late fall and winter of 1959–60 indicated the continued presence of substantial numbers of *tarsalis* females as well as other species.

METHODS. In early November 1959, several abandoned mines were checked for mosquitoes. Nineteen additional mines were examined in December with fewer mines visited monthly through March 1960. The mines were located within approximately a 5-mile radius of historic Virginia City at elevations ranging from about 5,000 to 6,200 feet. The mines examined were selected because of their accessibility and proximity to roads. This area (Virginia City-Silver City-Gold Hill) is undermined with innumerable tunnels, shafts, and pits. Shafts were not checked because of the danger involved in their examination. The mines varied in length from approximately 20 feet to more than a quarter of a mile in length and most of the tunnels were about 6 to 7 feet in height. Some mines contained large and small pools of water whereas others contained only seepage droplets of water on the walls and ceilings; many other mines were devoid of moisture.

Examination was done by flashlight. Periodically a sample of the mosquitoes was collected and identified in the laboratory. In 1959–60, temperature and relative humidity readings were taken with an aspirator-type psychrometer only in one month—January 1960. Generally, readings were taken in positive mines (mosquitoes present) as follows: just inside the mine entrance, in the immediate vicinity of the first *tarsalis* female, adjacent to other females, and then beyond the last ones if the mosquitoes did not occur at the end of the mine. In negative mines (no mosquitoes present), readings were taken just inside the mine entrance and at various intervals within.

In 1960–61, 16 mines were surveyed beginning in November 1960. Most were the same ones checked the year before. An additional five mines were checked in December 1960. All 21 mines were investigated monthly through February 1961, and then twice in March 1961. Temperature and relative humidity readings were taken monthly with a battery-operated psychrometer in a manner similar to that of the previous year. Searching for mosquitoes was done as in the previous year.

[1] In cooperation with the Nevada Agricultural Experiment Station, Reno, Nevada.

Some light-intensity readings were also recorded near females, as well as in portions of mines not containing mosquitoes, and in negative mines.

RESULTS. As shown in Table 1, only three species of mosquitoes were observed overwintering in abandoned mines throughout the two seasons. In 1959–60, *Culex tarsalis, Anopheles pseudopunctipennis franciscanus* McCracken, and *A. freeborni* Aitken comprised 93, 6, and 1 percent, respectively, of the total mosquitoes observed. The numbers of *tarsalis* seen in any one mine ranged from 0 to 145. Substantial numbers of *tarsalis* females were still present in late February (102 in one mine) whereas the mine populations were greatly reduced in early March. These reductions occurred after 2 days (March 5 and 6) when the temperature in the general area rose above 50° F. The lowest outside temperatures (° F.) recorded at Virginia City by the U. S. Weather Bureau were as follows: November, 27°; December, 12°; January, 4°; February, 11°; and March, 20°.

In 1960–61, *Culex tarsalis, Anopheles pseudopunctipennis franciscanus,* and *A. freeborni* comprised 88, 11, and 1 percent, respectively, of the mosquitoes present durthe observation period (Table 1). The highest number of *tarsalis* recorded in any mine was 75. In general this species was less abundant and *A. pseudopunctipennis franciscanus* more abundant than in the previous year. During the same month in the two observation periods, several mines contained almost exactly the same numbers and species. A sharp drop in numbers of *tarsalis* females was noted in mid-February. For several consecutive days prior to the February sampling, the temperature exceeded 50° F. Few *tarsalis* were present in March. The coldest outside temperatures (° F.) recorded by the U. S. Weather Bureau for Virginia City were as follows: November, 20°; December, 10°; January, 17°; February, 15°; and March, 20°. During both 1959–60 and 1960–61, *tarsalis* females were observed in 112 of 178 times that all mines were visited. None of the samples of the overwintering mosquitoes were blood-engorged and all possessed fat bodies.

The temperature and relative humidity data for both seasons are given in Table 2

TABLE 1.—Mosquitoes observed in abandoned mines from November 1959 to March 1960 and November 1960 to March 1961, near Virginia City, Nevada

Date observed	Number of mines visited	Number of mines with mosquitoes	Species			Average number per mine
			Culex tarsalis	*Anopheles pseudo-punctipennis franciscanus*	*Anopheles freeborni*	
1959						
Nov.	2	2	37	0	5	21.0
Dec.	21	14	309	27	4	16.2
1960						
Jan.	16	10	236	22	0	16.1
Feb.	8	5	139	1	0	17.5
March	10	4	25	0	0	2.5
Nov.	16	14	270	14	2	17.9
Dec.	21	18	283	39	2	15.4
1961						
Jan.	21	17	233	36	4	13.0
Feb.	21	14	113	20	3	6.5
March 8	21	11	37	4	0	1.9
March 30	21	3	2	0	1	0.1

and show an excellent correlation between the presence of *tarsalis* females in the mines and relative humidity. Positive mines consistently possessed higher relative humidities than did negative mines. Within a positive mine, relative humidity readings were considerably higher in the immediate vicinity of *tarsalis* females than in those portions uninhabited by this species. Relative humidities in some positive mines ranged up to 100 percent. Lesser differences were observed in the temperature readings taken in positive and negative mines. Females were never noted in portions of mines with temperatures above 60° F., and were usually absent just inside the entrances where temperatures usually averaged about 48–49°. Mosquitoes were seldom noted farther than several hundred yards within the mines since in general temperatures tended to increase from the entrance toward the end of the mines.

The greatest numbers of *tarsalis* females and *Anopheles* spp. were generally observed on ceilings of abandoned mines, with relatively few along the sides. The *Anopheles* spp. were especially prone to fly at a slight disturbance; *tarsalis* females were not so easily disturbed but could be induced to fly within the mines. During winter months some females were also seen leaving the mines. Light intensities did not appear to be an important factor in the presence or absence of *tarsalis* females nor in their particular placement.

DISCUSSION. Conditions for true hibernation as proposed by Blackmore and Winn (1956), that is, accumulation of fat body, absence of feeding and males, outside temperatures prohibitive for survival, and quiescence, were generally present for *tarsalis* populations in the abandoned mines. As mentioned previously, some *tarsalis* were prone to fly when disturbed and thus were not always completely quiescent. If temperatures in the vicinity of female *tarsalis* were adversely affecting the successful overwintering of these populations in the mines, surely the females would have sought the coolest portions, which were those adjacent to the entrance and near the floors. Our many observations indicated that this did not happen. Our readings undoubtedly represent the highest daily temperatures occurring in abandoned mines since these mines were always checked from about 10 a.m. to 3 p.m. and cooler temperatures must prevail during the remainder of the day.

The writer prefers to believe that in early March of 1960 and early February of 1961 the reductions that occurred in overwintering *tarsalis* populations as well as in *Anopheles* spp. were caused by the mosquitoes leaving the abandoned mines for lower elevations and not by mortality. In Utah, Dow *et al.* (1956) reported sim-

TABLE 2.—Mean temperatures (° F.) and relative humidities (R.H.) of abandoned mines in relation to the presence or absence of female *Culex tarsalis*

	Negative mines (no mosquitoes)		Positive mines (with mosquitoes)					
			Immediate vicinity of females		Females absent		Average	
Date observed	Temperature	R.H.	Temperature	R.H.	Temperature	R.H.	Temperature	R.H.
1960								
Jan.	50	41	50	66	51	64	50	65
Dec.	49	39	52	63	52	52	52	58
1961								
Jan.	53	32	51	57	51	41	51	51
Feb.	54	40	51	64	51	55	51	60
March	51	41	49	60	51	57	50	58
Average	53	39	51	62	51	54	51	58

ilar reductions in February and blamed them on mortality but admitted that increased temperatures might have forced the mosquitoes to leave.

The first breeding of *tarsalis* in the general area of the adjacent valleys was noted on April 15, 1959, April 6, 1960, and April 8, 1961. During the unaccounted-for time between their departure from the mines and breeding, the females must have had some temporary resting site. Overwintering mosquitoes were undoubtedly much fewer than normal during these two winters since the paucity of snow pack in the mountains in 1958–59 and 1959–60 greatly reduced both permanent and temporary breeding sites.

Summary. A 2-year survey of abandoned mines indicated the presence of only three species of overwintering female mosquitoes: *Anopheles freeborni, A. pseudopunctipennis franciscanus,* and *Culex tarsalis.* During each of the 2 years *tarsalis* represented 93 and 88 percent, respectively, of the total mosquitoes observed in the mines. During both years a sharp reduction in *tarsalis* occurred in early March and February but was attributed to an exodus from the mines rather than mortality. The maximum number of *tarsalis* females noted in any one mine during 1959–60 and 1960–61 was 145 and 75, respectively.

Temperatures were slightly lower and relative humidities much higher in mines where mosquitoes were present than in those where no mosquitoes were found. Average temperatures were also slightly lower and relative humidities somewhat higher in portions of the mines where *tarsalis* females were present than in those parts where they were not.

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The **FLORIDA ANTI-MOSQUITO ASSOCIATION** Annual Meeting will be held on April 29th and 30th and May 1st and 2d, 1962 in Silver Springs, Florida.

The Annual Meeting of the **VIRGINIA MOSQUITO CONTROL ASSOCIATION** will be held on February 23d in Norfolk or vicinity. Details are lacking at the time of this writing.

A BIOASSAY METHOD AND RESULTS OF LABORATORY EVALUATION OF INSECTICIDES AGAINST ADULT MOSQUITOES [1, 2, 3]

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INTRODUCTION. In dealing with the insecticide resistance problem, the World Health Organization (W.H.O.) initiated in 1960 a long-term project for the comparative toxicological evaluation of potential insecticidal compounds. Under the terms of this project, a number of cooperating pesticide manufacturers submit outstanding new compounds to the W.H.O. who, in turn, channel them through several laboratories in various parts of the world, where they are screened against susceptible and resistant house flies and mosquitoes, evaluated for mammalian toxicity, studied under conditions of tropical storage, and tested for field performance. This is probably the first international cooperative effort in the methodical evaluation of insecticides, and the results will undoubtedly prove of immense benefit not only in the control of vector species but also in all phases of insect control by chemicals. This project is particularly timely in view of the increasing concern over the development of insecticide resistance which might jeopardize the success of mosquito eradication campaigns in various countries. By early 1960, the number of species of anopheline mosquitoes which are reported to have developed resistance to DDT and/or dieldrin had risen to 28 (Brown 1961). The assignment of stage I (laboratory screening) of this project to the University of California, Riverside, has presented the writers with the need for selecting or devising a bioassay method for testing the candidate compounds against adult mosquitoes.

Testing methods for house flies and mosquito larvae are fairly well standardized and generally accepted. There is, however, no satisfactory method for evaluating compounds against adult mosquitoes. Methods already published are designed mainly for small scale tests, or for the assay of resistance development. The present project calls for a method permitting the rapid evaluation of a large number of compounds of diverse chemical nature, with standardized equipment, solvent, and handling procedure. The applicability of the published methods to the present problem was thoroughly explored. The topical application method, which undoubtedly yields reproducible results independent of behavioral patterns of the test species, has been tried on mosquitoes (Ludvik, 1953; Hadaway and Barlow 1956; Ungureanu 1958) but never gained much popularity. One drawback of this method is that the insects must be handled individually, which, in the case of such fragile forms as mosquitoes, produces variable control mortalities dependent on the care and experience of the operator. Treatment by this means is of necessity slow. Other disadvantages are the ill effects of prolonged anesthesia, and the toxicity of acetone when used in volumes necessary (over 0.5 microliters per insect) for satisfactory topical application. Attention was, therefore, concentrated on contact methods of exposure.

Busvine and Barnes (1948) described a method involving exposure of mosquitoes to filter paper treated with acetone solu-

[1] Paper Number 1325, Citrus Research Center and Agricultural Experiment Station, University of California, Riverside.

[2] The authors are grateful to Mrs. Marian Fairchild and Mrs. Eula Ryan for their able laboratory assistance in connection with the rearing and testing of mosquitoes.

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tions of insecticides. The insects were confined between two petri dishes each lined with a filter paper. This method was subsequently modified (Busvine and Nash 1953) mainly by substituting a refined mineral oil for acetone as solvent. The mosquitoes were enclosed in cylinders made by rolling up the filter paper and closing the ends with glass discs. A roughly similar procedure was described by Fay *et al.* (1953) for the measurement of resistance to DDT. These authors utilized test chambers formed by rolling up treated papers in cylinders, and covering the ends with screen-wire caps. For treatment, the papers were dipped in a xylene solution of DDT, then drained vertically and dried in a horizontal position for 24–72 hours. Mathis *et al.* (1959) utilized the more important features of the methods of Busvine and Nash (1953) and of Fay *et al.* (1953), and designed an excellent testing kit, which has been incorporated in the widely used W.H.O. testing method for the measurement of insecticide resistance in adult mosquitoes. While the method ultimately decided upon as satisfactorily meeting the requisites of a laboratory screening program utilizes the essential principle of the W.H.O. testing method, it contains several modifications involving the type of solvent used, the exposure chamber and the handling procedure. The tests which led to these modifications are briefly discussed below.

Tests Leading to Development of Present Method. The present tests were performed on *Culex pipiens quinquefasciatus* Say, and *Anopheles albimanus* Wied. *C. p. quinquefasciatus* has been reared at the University of California, Riverside, for over 12 years without intentional exposure to chemicals, and is of normal susceptibility to the common insecticides. *A. albimanus* originated in Panama City, Panama, and is highly resistant to dieldrin (LC_{50} larvae 0.4 p.p.m., adults $>$ 100 cm^2). *C. p. quinquefasciatus* is maintained at a constant temperature of 75° F. and 60 percent r.h. and *A. albimanus* at 80° F. and 60 percent r.h. In both instances, a 12-hour photoperiod with one hour of twilight at 12-hour intervals, is observed. Unless otherwise indicated, the tests were carried out with 2- to 3-day old insects at 74 ± 2° F. The sexes were not segregated. After treatment, the insects were held at 60° F. and 60 percent r.h. and mortality was determined 24 hours later.

Anesthesia. In tests involving flying insects, it is desirable that these be briefly anesthetized so that they may be counted accurately and transferred with the minimum of handling. Various anesthetizing agents such as ether, chloroform or carbon dioxide have been utilized in handling mosquitoes. Testing methods intended for field use, or involving only a small number of insects, are designed so as to eliminate the need of an anesthetic (W.H.O. testing kit). Ether has been used satisfactorily in small scale tests (Ungureanu 1958), but for large scale operations in which over 1000 mosquitoes are handled daily, the use of carbon dioxide presents certain distinct advantages: (1) It is available in cylinders from which it can be discharged through a pressure regulating valve, and piped into a "knock-out" chamber, a counting dish, or elsewhere in the laboratory for as long as necessary; (2) provided the mosquitoes are not exposed to excessively high concentrations of the gas, or for unduly long periods of time, they will tolerate carbon dioxide anesthesia without ill effects; (3) most insecticide screening laboratories are equipped with CO_2-dispensing facilities, so that the existing set-up may be utilized for mosquito testing without substantial changes.

Tarshis (1957) has kept *Aedes aegypti* (Linn.) under CO_2 anesthesia for 2 hours without ill effects. CO_2 anesthesia was also employed in contact and topical application tests involving *Anopheles quadrimaculatus* Say (Ludvik *et al.* 1951; Ludvik 1953). The insects were placed in a CO_2 "knock-out" chamber (a battery jar) for 1 minute and then transferred to a counting dish through which a gentle stream of CO_2 was piped, and were exposed to the gas for periods varying from

3 to 30 minutes. One hour after this exposure, they were anesthetized again for 2 minutes and transferred to holding cages at 60° F. This double exposure to CO_2 was intended to simulate the type of anesthetization utilized in the method described later on in this paper. However, the 30-minute exposure tested is far beyond the few minutes required for normal handling.

After first exposures of 3, 10, 20 and 30 minutes for *C. p. quinquefasciatus* and 3, 5, 7, 10, 15, 20 and 30 minutes for *A. albimanus* there was 100 percent recovery of all specimens. After second exposure of 2 minutes for each of the above lots there was 95 percent to 100 percent recovery. Twenty-four hours after exposure the percentages of mortality, corresponding to the times of the first exposure periods tested above, were, respectively 0, 0, 3, 0 for *C. pipiens quinquefasciatus* and 8, 5, 0, 10, 5, 0 and 10 for *A. albimanus*.

Solvents. In dealing with hundreds of organic compounds of varying types of solubility, acetone has proved to be the most suitable solvent (Metcalf 1958), and standard w/v acetone solutions are used throughout the screening programs of this and most other laboratories. However, since oil has been suggested as solvent in filter paper contact tests, it was desirable to study some of the relative features of the two solvents.

Tests were carried out on the performance of insecticide deposits from acetone solution on dry filter paper, as compared with similar deposits on filter paper pretreated with various concentrations of refined mineral oil. Using the test method described below, the results in Table 1 were obtained with DDT, malathion and *m*-isopropylphenyl *N*-methylcarbamate. These indicate that the toxicity of DDT was slightly increased by low concentrations of oil but later decreased considerably as the concentration of oil was increased. Conversely, the toxicity of the carbamate was reduced by low concentrations of oil, and subsequently increased beyond the initial toxicity of the compound. The toxicity of malathion showed a steady increase with increasing concentration of oil.

To determine the effect of oil on insecticide deposits on glass surfaces, open-ended glass vials were obtained in which the ends were slightly constricted so that the vial could hold at least 1 ml. of solution when lying on its side. One-half ml. of acetone solution of the insecticide and one-half ml. of acetone solution of the oil were introduced and the solvent was evaporated by rotating the vials on an electrically operated roller mill. The results (Table 2) show that while the toxicity of malathion and carbamate was somewhat increased in the presence of oil, that of DDT was again decreased. In the tests with malathion and carbamate, there appeared to be an optimum concentration of oil beyond which the performance of the insecticide was reduced. Hoskins *et al.* (1952) suggested that high concentrations of oil affect adversely the normal activity of house flies on glass surfaces

TABLE 1.—Toxicity of insecticides on filter paper impregnated with various concentrations of refined mineral oil, as measured against *Culex pipiens quinquefasciatus*

Compound and concentration	24-hour percent mortality						
	Concentration of oil (γ/cm.^{2})						
	0	1.6	4.7	16	47	160	470
DDT 7.8 γ/cm.^{2}	67	71	76	84	60	12	0
Malathion 6.3 γ/cm.^{2}	10	12	30	62	59	63	66
AC 5727* 0.24 γ/cm.^{2}	48	18	28	54	71	70	84

* *m*-isopropylphenyl *N*-methylcarbamate.

TABLE 2.—Toxicity of insecticides in glass vials, treated with various concentrations of refined mineral oil, as measured against *Culex pipiens quinquefasciatus*

Compound and concentration	24-hour percent mortality					
	Concentration of oil (γ/cm.²)					
	0	0.19	1.9	5.7	19	95
DDT 0.19 γ/cm.²	59	49	49	24	24	6
Malathion 0.038 γ/cm.²	75	65	88	85	48	8
AC 5727* 0.0019 γ/cm.²	75	78	90	78	100	48

* *m*-isopropylphenyl *N*-methylcarbamate.

and consequently the extent of their exposure to the toxic residue. Such an effect was apparent in the tests reported here with mosquitoes. It is obvious from these data that the use of oil in comparative screening tests would enhance the performance of some insecticides while suppressing that of others, thus creating an erroneous impression of the intrinsic toxicity of the pure insecticide deposit. These difficulties with oil-insecticide surfaces led us to discard the idea of glass surfaces in favor of impregnation of the compound on filter paper from acetone. Application of the insecticide to filter paper rather than to bare glass presents certain distinct advantages: the paper can be treated more evenly by saturating it with the volatile solvent, and there is no problem with supersaturated droplets or induced crystallization; the insects have a firm foot hold and thus excessive activity, which in bare glass vials results in considerable physical injury to the test insects, is avoided; exposure is for the most part through the tarsi, thus approaching the type of contact obtained under field conditions.

Interval between Treatment of Paper and Exposure of Insects. In the W.H.O. testing kit the oil-treated papers may be used several months after impregnation (Busvine 1958; Mathis *et al.* 1959). In the present work it was observed that when acetone is used as solvent there is a negligible loss in toxicity during the first four hours (Table 3), which, however, becomes greater with time, at rates varying with the different compounds. The percentage of loss in toxicity as measured against *Culex p. quinquefasciatus* adults, 12 days after application was as follows: dieldrin 20; parathion 29; *m-sec*-butylphenyl *N*-methylcarbamate 39; *m*-isopropylphenyl *N*-methylcarbamate 41; and DDT 57. In practice, it is recommended that insects be exposed to the toxic residue within 2 hours after application. Numerous tests run within this period have shown no greater variation than what may be attributable to day-to-day differences in the susceptibility of the test population.

Exposure Chambers. Various types of exposure chambers were investigated in an effort to find one which would ensure virtually continuous contact of the insects with the treated surface. These included petri dishes, combinations of half petri dish and powder funnel, glass tubes open at both ends and shell vials open at one end.

TABLE 3.—Persistence of activity of insecticides applied to filter paper from acetone solutions, as measured against *Culex pipiens quinquefasciatus*

Compound	Concentration (γ/cm.²)	24-hour percent mortality*						
		Interval between treatment of filter paper and exposure of insects (hours)						
		.0.25	0.5	1	1.5	2	3	4
DDT	11	88	90	83	83	82	88	78
Baytex	1.26	100	98	87	97	88	92	93
AC 5727**	0.28	85	85	77	75	82	83	82

* Average of 3 replications.

** *m*-isopropylphenyl *N*-methylcarbamate.

Petri dishes and petri dish-powder funnel combinations were inconvenient and frequently resulted in high insect mortality during handling. When petri dish-powder funnel combinations were used, the mosquitoes tended to settle on the walls of the funnel rather than on the treated dish or filter paper surface. This avoidance reaction was more pronounced during the late afternoon and early evening hours. Coating the funnel with a light film of vaseline forced the mosquitoes to remain on the insecticide-treated surface, but this procedure was rather tedious. Shell vials (2.1 x 8.4 cm.) were found to be the most satisfactory: they can be handled in groups without danger of crushing the insects, they can be accommodated in relatively small anesthetization chambers, and are readily available at reasonable cost. Treated filter paper can easily be rolled up and placed into the vial. The treated area thus comprises 89 percent of the total area of the exposure chamber, the two ends of the vial remaining untreated. As the insects move about inside the vial, they are exposed to the residue. The very rare mosquito which remains on the untreated surface throughout the period of exposure does not materially affect the LC_{50} or LC_{90} values obtained.

Holding Chambers. Davidson (1958) used ½-pint unwaxed paper cups fitted with a net cover, as post-exposure holding chambers. In the present tests, such cups were found very convenient, especially when fitted with a snap-on transparent plastic cover, which permits unobstructed examination of the insects. The cups are provided with a small piece of cotton roll moistened with 10 percent sucrose solution and are discarded after use.

THE TEST PROCEDURE. The pupae are collected either with a hand strainer, a pupal separator, or by some other means, depending on the species. Approximately 300 pupae are placed in emergence cages consisting of 1-gal. ice cream containers with cloth sleeves and a screen end. The pupae are moved daily to new emergence cages so that mosquitoes of known, uniform age are available. A waxed paper cup filled with cotton soaked with 10 percent sucrose solution is placed in each cage to provide nourishment for the adults. Since it is more convenient to avoid blood feeding, the adults are used on the second or third days after emergence. The sexes are not segregated.

The insecticide is applied in standard w/v acetone solution to Whatman No. 2 9-cm. diameter filter paper placed horizontally on pin points. One ml. of the liquid (which completely saturates the paper) is pipetted onto the paper spirally and allowed to evaporate completely. After about 5 minutes, a section approximately 0.5 cm. wide at the widest point is trimmed off one side of the paper and the latter is rolled up into a shell vial (2.1 x 8.4 cm.) lining the sides almost completely. For the sake of uniformity, the insects are exposed to the treated paper within one hour and in no case later than two hours after treatment.

The insects are anesthetized for 1–2 minutes by placing the emergence cage in the "knock-out" chamber, separated into groups of 20 with a camel's hair brush in a counting dish through which CO_2 is being piped, and transferred to the test vials with an aspirator. The vials are covered at the open end with cheesecloth, and placed flat on the side under standard conditions of temperature (74° F.) and lighting. The insects normally recover within 5 minutes from the onset of anesthesia. Slight jarring of the vials speeds up the recovery period. One hour after initiation of exposure the insects are lightly anesthetized and transferred into holding cages (described above). These are kept in the dark at 60° F. and 60 percent r.h. to minimize activity, and mortality is determined after 24 hours.

In standard practice, the compounds are tested at 0.1 percent concentration (16 $cm.^2$), and those producing less than 50 percent mortality are excluded. The remaining are tested further for the determination of LC_{50} values. Each LC_{50} is

TABLE 4.—Dosage mortality data for Baytex and DDT against *Culex pipiens quinquefasciatus* adults

Compound	Concentration (γ/cm.^{2})	Percentage mortality in replications[a] I	II	III	IV	V	Average	LC_{50} (γ/cm.^{2})	Slope
Baytex									
	0.47	10	0	0	0	0	2	..	..
	0.63	20	25	0	0	5	10	..	..
	0.78	30	60	5	0	5	10	..	..
	0.94	85	65	5	35	20	42	..	..
	1.26	95	85	35	40	45	60	..	..
	1.6	95	95	40	50	85	73	1.1	5.2
DDT									
	2.4	..	5	5	10	0	5	..	..
	3.1	10	10	5	20	5	10	..	..
	4.7	45	35	10	15	25	26	..	..
	6.3	..	..	35	30	35	33	..	..
	7.8	85	80	40	40	60	61	..	..
	12.6	..	..	..	60	90	75	6.9	3.3

[a] Performed on different dates.

based on about 5 concentrations of the insecticide replicated on 3 or 4 different days.

A skilled person may treat approximately 1000 mosquitoes within 3 to 4 hours. The cost of supplies, mostly cups and lids, is estimated at 73 cents per LC_{50} determination, if 5 concentrations are replicated 4 times.

Although the present method was designed on the basis of tests performed on *C. p. quinquefasciatus* and *A. albimanus,* it is believed to be applicable to other species of mosquitoes as well.

RESULTS AND DISCUSSION. A set of typical data obtained with the method described, is given in Table 4. The steepness of the slope of the dosage-mortality regression lines manifests a high degree of sensitivity and correlation between concentration and mortality. Table 5 shows the percentage of mortality and the coefficient of variation for replicated treatments of DDT. The variation is satisfactorily small and comparable to that encountered in topical application data with house flies. Day to day variation is likely to be greater (see Table 4) and will depend largely on the degree of standardization of the rearing method and on the homogeneity of the test population.

The importance of several other sources of error in technique was also investigated. Varying the period of exposure to CO_2 immediately prior to exposure to the toxic residue did not significantly alter the degree of mortality (Table 6). Increasing the length of exposure to the residue from 45 to 75 minutes resulted in corresponding increases in mortality (Table 7).

TABLE 5.—Variation in mortality of adult mosquitoes in replicated treatments of same date

Species	Compound and concentration	Percentage of mortality (Replications)					Ave.	Standard deviation	Coefficient of variation
Culex pipiens quinquefasciatus	DDT ($3.1\cdot\gamma$/cm.^{2})	65	65	75	55	65	63.5	6.25	0.098
		60	65	70	60	55			
Anopheles albimanus	DDT ($0.24\ \gamma$/cm.^{2})	25	20	25	20	30	24	4.18	0.174

TABLE 6.—Effect of CO_2 anesthesia immediately preceding exposure to toxic residue, on mortality of *Culex pipiens quinquefasciatus*

Length of exposure to CO_2 (minutes)	24-hour percent mortality* with insecticide indicated: DDT (7.8 γ/cm.²)	AC 5727** (0.24 γ/cm.²)	Control
5	28	43	..
8	17	40	..
12	22	27	..
20	38	57	0
28	53	53	2
36	28	30	3

* Average of three replications.
** *m*-isopropylphenyl *N*-methylcarbamate.

TABLE 7.—Influence of length of exposure to insecticide-treated filter paper on mortality of *Culex pipiens quinquefasciatus* adults

Length of exposure to insecticide residue (minutes)	24-hour percent mortality* with insecticides indicated: DDT (4.7 γ/cm.²)	Baytex (0.78 γ/cm.²)	AC 5727** (0.2 γ/cm.²)
45	57	58	38
50	48	73	45
55	63	83	50
60	70	90	58
65	68	93	73
70	68	95	77
75	75	95	63

* Average of three replications.
** *m*-isopropylphenyl *N*-methylcarbamate.

Holding the treated insects at 80° F. instead of 60° F. produced lower mortalities (Table 8), indicating a more efficient detoxication mechanism for the particular insecticide at the higher temperature. These results point out the great importance of rigidly standardized procedures in an insecticide evaluation method.

TABLE 8.—Influence of post-exposure holding temperature on mortality of adult mosquitoes

Species	Treatment	24-hr. percent mortality: 60° F.	80° F.
Culex pipiens quinquefasciatus	AC 5727* (0.2 γ/cm.²)	42	34
	Control	0	2
Anopheles albimanus	AC 5727* (0.16 γ/cm.²)	33	17
	Control	0	0

* *m*-isopropylphenyl *N*-methylcarbamate.

As a further test of the validity of the present method, the results obtained with DDT, Baytex, *m*-isopropylpheny *N*-methylcarbamate and Dow Chemical (*O*-methyl *O*-(2,4,5-trichlorophenyl) ethylphosphoramidate) by the contact and topical application methods were compared (Table 9). In the topical treatments, the materials were applied to the nota of the test mosquitoes in 0.3 microliter drops with a micrometer-driven microsyringe. CO_2 exposure during treatment was kept at a minimum by turning the gas on and off as necessary, and control mortality due to CO_2 and acetone was less than 5 percent. The results indicate a very close agreement in the relative toxicity of DDT and Baytex by the two methods. The small divergence in the results obtained with *m*-isopropylphenyl *N*-methylcarbamate may

TABLE 9.—Susceptibility of *Culex pipiens quinquefasciatus* to various insecticides as determined by the contact and topical application methods

Compound	Topical [a]: LD_{50} (γ/gm.)	Slope	Contact: LC_{50} (γ/cm.²)	Slope
DDT	6.99	3.4	6.9	3.3
Baytex	1.11	3.6	1.1	5.2
AC 5727 [b]	0.09	2.8	0.2	5.6
Dow Chemical [c]	39.7	3.3	>16	..

[a] Average weight 1.26 mg./mosquito.
[b] *m*-isopropylphenyl *N*-methylcarbamate.
[c] *O*-methyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidate.

TABLE 10.—Comparative toxicity of several insecticides to larvae and adults of *Culex quinquefasciatus* and *Anopheles albimanus*

	Compound	Chemical name	Larval LC_{50} (p.p.m.)		Adult LC_{50} (γ/cm.^{2})	
			Culex	*Anopheles*	*Culex*	*Anopheles*
1.	DDT	2,2-*bis* (*p*-chlorophenyl) 1,1,1-trichloroethane	0.07	0.015	6.9	0.23
2.	Lindane	gamma-hexachlorocyclohexane	0.025	0.21	0.14	0.2
3.	Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	0.0078	0.4	0.95	>16
4.	Allethrin	*dl*-allylrethronyl *dl-cis-trans*-chrysanthemate	0.14	0.96	11.0	3.3
5.	LB–95–61	2,4-dimethyl benzylchrysanthemate	0.09	0.1	1.9	1.7
6.	Malathion	*S*-(1,2-dicarbethoxyethyl) *O,O*-dimethylphosphorodithioate	0.081	0.12	5.9	4.4
7.	Ethyl malathion	*S*-(1,2-dicarbethoxyethyl) *O,O*-diethylphosphorodithioate	0.3	0.43	>16	ca. 10
8.	Bayer 16450	*O,O*-diethyl-1-acetyl-1-carbethoxy methylphosphonate	0.11	0.28	5.4	1.8
9.	Dibrom	1,2-dibromo-2,2-dichloroethyl dimethylphosphate	0.11	0.16	1.1	0.92
10.	Guthion	3-(4-ketobenzotriazine)-methyl *O,O*-dimethylphosphorodithioate	0.025	0.22	>16	>16
11.	DDVP	*O,O*-dimethyl-*O*-2,2-dichlorovinyl phosphate	0.075	0.1	0.088	0.094
12.	Bayer 24882	*O*-methyl *O*-(1-chloromethylethyl)-*O*-2,2-dichlorovinyl phosphate	0.036	0.1	1.0	1.2
13.	Bayer 22684	*O*-methyl *O*-2-chloroethyl-*O*-2,2-dichlorovinyl phosphate	0.07	0.17	0.4	0.3
14.	EPN	ethyl-4-nitrophenyl phenylphosphonothionate	0.0045	0.0084	14.0	12.0
15.	Methyl parathion	*O,O*-dimethyl *O*-4-nitrophenyl phosphorothionate	0.018	0.096	0.33	0.52
16.	Parathion	*O,O*-diethyl *O*-4-nitrophenyl phosphorothionate	0.0032	0.0047	1.2	0.46
17.	*n*-propyl parathion	*O,O*-di-*n*-propyl *O*-4-nitrophenyl phosphorothionate	0.011	0.064	7.2	11.0
18.	Dicapthon	*O,O*-dimethyl *O*-(2-chloro-4-nitrophenyl) phosphorothionate	0.027	0.093	5.7	7.1
19.	Chlorthion	*O,O*-dimethyl *O*-(4-nitro-3-chlorophenyl) phosphorothionate	0.026	0.13	8.0	ca. 16.0
20.	Ethyl Dicapthon	*O,O*-diethyl *O*-(2-chloro-4-nitrophenyl) phosphorothionate	0.0062	0.02	2.2	2.6
21.	Dow	*O*-propyl *O*-(4-nitrophenyl)methylphosphoramidothioate	0.023	0.062	ca. 16	>16
22.	Bayer S-5660	*O,O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothionate	0.0058	0.011	2.8	3.3
23.	Bayer 30237	*O*-methyl *O*-4-methylthiophenyl methylphosphonothionate	0.0034	0.015	0.47	0.68
24.	Bayer 38107	*O*-methyl *O*-4-methylthiophenyl ethylphosphonothionate	0.0031	0.0096	0.38	1.5
25.	Bayer 38104	*O*-ethyl *O*-4-methylthiophenyl methylphosphonothionate	0.0043	0.007	0.98	0.64
26.	Bayer 32384	*O,O*-dimethyl *S*-4-methylthiophenyl phosphorodithioate	0.0086	0.025	11.0	7.7
27.	Bayer 34098	*O*-(3-methyl-4-methylthiophenyl)dimethylphosphinothionate	0.025	0.12	6.2	9.4
28.	Bayer 33333	*O*-methyl *O*-(3-methyl-4-methylthiophenyl)methylphosphonothionate	0.011	0.024	10.0	3.2
29.	Bayer 38108	*O*-methyl *O*-(3-methyl-4-methylthiophenyl)ethylphosphonothionate	0.0027	0.012	0.78	1.0
30.	Baytex	*O,O*-dimethyl *O*-(3-methyl-4-methylthiophenyl)phosphorothionate	0.0045	0.016	1.1	1.2
31.	Bayer 29492	*O,O*-diethyl *O*-(3-methyl-4-methylthiophenyl)phosphorothionate	0.011	0.015	1.7	2.7
32.	Bayer 34042	*O*-ethyl *O*-(3-methyl-4-methylthiophenyl)*N*-methylphosphoramidothionate	0.0035	0.0095	7.6	6.0
33.	Bayer 30468	*O*-ethyl *O*-(*p*-ethylthiophenyl)methylphosphonothionate	0.0028	0.011	1.0	0.33

TABLE 10.—Continued

	Compound	Chemical name	Larval LC_{50} (p.p.m.)		Adult LC_{50} (γ/cm.^{2})	
			Culex	*Anopheles*	*Culex*	*Anopheles*
34.	Bayer 29491	*O,O*-dimethyl *O*-(3-chloro-4-methylthiophenyl)phosphorothionate	0.0018	0.0044	1.0	1.4
35.	Bayer 37343	*O,O*-diethyl *O*-(3,5-dichloro-4-methylthiophenyl)phosphorothionate	0.0015	0.0016	1.2	1.3
36.	Bayer 30554	*O*-methyl *O*-(4-methylsulfinylphenyl)methylphosphonothionate	0.027	0.11	3.4	7.2
37.	Ronnel	*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl)phosphorothioate	0.03	0.066	0.61	2.8
38.	Dow	*O*-methyl *O*-(2,4,5-trichlorophenyl)methylphosphoramidothioate	0.03	0.23	>16	>16
39.	Dow	*O*-methyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidothioate	0.02	0.15	12	>16
40.	Dow	*O*-methyl *O*-(2,4,5-trichlorophenyl)isopropylphosphoramidothioate	0.025	0.22	12	>16
41.	Dow	*O*-ethyl *O*-(2,4,5-trichlorophenyl)methylphosphoramidothioate	0.043	0.22	>16	16
42.	Dow	*O*-ethyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidothioate	0.025	0.096	9	11
43.	Dow	*O*-isopropyl *O*-(2,4,5-trichlorophenyl)methylphosphoramidothioate	0.032	0.052	>16	>16
44.	Dow	*O*-isopropyl *O*-(2,4,5-trichlorophenyl)ethylphosphoramidothioate	0.038	0.028	>16	>16
45.	Dow	*O*-isopropyl *O*-(2,4,5-trichlorophenyl)isopropylphosphoramidothioate	0.043	0.023	12	13
46.	Dow	*O*-methyl *O*-(2,4-dichlorophenyl)ethylphosphoramidothioate	0.064	0.064	9.5	15

indicate a differential in the rates of absorption of the compound by the two alternative routes of entry.

Over 250 compounds have already been tested by the present method and the results have been made available to W.H.O. Some of the more active mosquito adulticides and larvicides encountered in the present work are given in Table 10. Data on several commercially available insecticides have been included for comparison. The results indicate the outstanding larvicidal activity of a number of methyl- and chloro-substituted *p*-methylthiophenyl or *p*-ethylthiophenyl phosphorus esters (compounds 23–25, 29, 30, 32–35), and also the high adulticidal activity of certain aryl *N*-methylcarbamates. The latter have been listed in other papers (Georghiou and Metcalf 1961, 1962). Similarly, cyclodiene compounds are dealt with separately (Metcalf *et al.* 1962). Several chloro-substituted phosphoramidothioates (compounds 38–46) which have been found to be remarkably toxic to susceptible and resistant strains of the house fly (unpublished data), are rather ineffective against mosquitoes. It is interesting that some of the very active organophosphorus larvicides (compounds 14, 26, 32) are less active as adulticides in comparison with some aryl *N*-methylcarbamates (Georghiou and Metcalf 1961). Conversely, the latter show poor larvicidal and outstanding adulticidal activity. This evident lack of consistent correlation between larvicidal and adulticidal activity in a number of insecticides points out the need for evaluating candidate compounds against both stages of mosquitoes.

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ASSOCIATION NEWS

PROGRAM OUTLINE

FOR THE

JOINT MEETING OF THE AMERICAN MOSQUITO CONTROL ASSOCIATION AND THE TEXAS MOSQUITO CONTROL ASSOCIATION, MARCH 5–7, 1962, AT THE HOTEL GALVEZ, GALVESTON

March 4 (Sunday)

9:00 a.m. Meeting of the Board of Directors

6:30 p.m. Luncheon for AMCA Board, TMCA Officers and Committee

March 5 (Monday)

OPENING SESSION

Presiding: President, Texas Mosquito Control Assn.

9:00 a.m. Call to Order: William E. Bickley, President AMCA

9:15 a.m. Welcoming Addresses: Honorable Edward Schreiber, Mayor of the City of Galveston; P. J. Gurklis, Chairman, Mosquito Advisory Board, Brazoria County Mosquito Control District

9:45 a.m. Preliminary Business Meeting

10:15 a.m. Recess

10:30 a.m. Submitted Papers (8)

AFTERNOON SESSION

1:30 p.m. Symposium: *Newer Approaches to the Control of Mosquitoes*

Nematodes: H. E. Welch
Microsporidia: Wm. R. Kellen
Chemosterilants: Carroll N. Smith
Release of irradiated Aedes aegypti *males:* H. F. Schoof
Genetics: George Craig

3:30 p.m. Recess

3:45 p.m. Submitted Papers (7)

6:30 p.m. Trip to Falstaff Brewery—Barbecue

March 6 (Tuesday)

MORNING SESSION

8:30 a.m. Submitted Papers (7)

10:15 a.m. Recess

10:30 a.m. Invitational Paper: *Recent Developments in Studies of Reactions to Mosquito Bites:* A. S. West

11:00 a.m. Equipment Demonstration

AFTERNOON SESSION

1:30 p.m. Symposium: *Granular Insecticides and Their Use as Mosquito Control Agents;* Moderator: Mir S. Mulla

Physico-Chemical Factors Influencing the Effectiveness of Granular Insecticides: Mir S. Mulla

The Formulation and Application of Light-Weight Granular Mosquito Larvicides: Andrew J. Rogers

Factors Affecting Release of DDT from Granular Particles: Donald L. Sutherland

Problems in the Formulation of Granular Pesticides: James W. Young

Use of Granular Larvicides for Mosquito Control in California: Art F. Geib

The Use and Application of Granular Larvicides with a Compressed Air Gun: Jack H. Kimball

DDT Granules for Mosquito Control in Minnesota: Albert W. Buzicky

3:30 p.m. Recess

3:45 p.m. Final Business Meeting

6:30 p.m. Hospitality Hour

7:30 p.m. Buffet Dinner

March 6 (Tuesday)

Concurrent Trustees Session

Moderator: G. F. MacLeod

Morning Session

9:00 a.m. Panel Discussion: *Enabling Legislation — Variations, Strengths and Weaknesses in Various Regions*

Eastern Region: Spencer S. Marsh, Jr., Madison, New Jersey
Southern Region: William Dryden, Ft. Myers, Florida
Middle Plains Region: George W. Mathews, St. Paul, Minnesota
Mountain States Region: John Marsh, Ogden, Utah
Southwest Region: P. J. Gurklis, Lake Jackson, Texas
Western Region: Marion C. Bew, Marysville, California

12:00 Noon Lunch
Speaker: Richard W. Fay—*Impact of the Space Age on Mosquito Problems*

2:00 p.m. Panel Discussion: *Policy Problems Confronting Directors and Trustees*

Public Relations and Education: Don Rees, Salt Lake City, Utah
Budgets and Finance: Bailey B. Pepper, New Brunswick, N. J.
Salaries and Wages: James Lesparre, Midlothian, Illinois
Insurance and Liabilities: Francis Creadon, Lyons, Illinois

3:30 p.m. Adjourn

March 7 (Wednesday)

Morning Session

8:30 a.m. Submitted Papers (6)

9:45 a.m. Recess

10:00 a.m. Symposium: *Water Management in Mosquito Control*

The Use of Trencher in Drainage Projects: Brice Johns, South Cook County, Illinois, MAD
Pilot Experiments in Water Management in Louisiana: E. S. Hathaway, Louisiana Mosquito Control Association
Water Management Structures in New Jersey: Lester W. Smith, Middlesex County Mosquito Extermination Commission
Water Impoundments and Other Types of Water Management in Florida: E. A. Philen, Florida State Board of Health
Water Management as Applied to Agricultural and Dairying Practices: W. D. Murray, Delta Mosquito Abatement District, California

Afternoon Session

1:30 p.m. Submitted Papers (12)

3:45 p.m. Board of Directors Meeting

SCIENTIFIC NOTES

IMPROVED TECHNIQUES FOR THE LABORATORY REARING OF *Aedes aegypti* (LINN.)

JOHN E. PORTER, GEORGE KOZUCHI AND MARTIN J. KUCK [1]

Various methods have been utilized in rearing *Aedes aegypti* in the laboratory. Optimum temperature and humidity conditions have been amply defined by Horsfall (1955), Trembley (1955), the United States Department of Agriculture (1955) and Christophers (1960). Conditions necessary to achieve maximum egg deposition and to maintain healthy colonies of larvae have been well described by these authors.

Rearing containers and cages for adults show considerable variation in shape, size and complexity depending upon the ultimate purpose for which the specimens are being reared. Trembley, Chao (1959) and Christophers propose the use of various sizes and shapes of cages covered with screen netting and/or clear plastic; Pollard (1960) adds a built-in animal restrainer to a cage of the type described by Trembley while Micha Bar-Zeev and Galun (1960) suggest a more intricately designed cage to prevent the escape of infected adults.

Many investigators have indicated satisfaction with the feeding devices and nutritional ingredients used for maintaining colonies of adult *aegypti* and these have become more or less standardized. It is, however, understandable that each investigator has the right to choose, modify or develop those procedures most satisfactory to his particular needs. It was because of problems peculiar to the investigations undertaken by the authors that necessity dictated the development of certain modifications in these standard procedures which we hope may be of value to other workers.

Adult female *aegypti* require both blood meals and a sugar or honey-water solution as an alternate food while the male mosquito will survive on the sugar or honey-water solution alone. In the warm and humid conditions often characteristic of an insectary, preparations of sugar or honey solution very quickly ferment and mold. Pads or wicks of cotton soaked in this solution need to be frequently turned over, remoistened or replaced. Then too, these cotton pads or wicks are also attractive oviposition sites for the gravid females.

It is evident that we need (1) a means of preventing fermentation and molding of the honey solution, (2) a container which will prevent rapid evaporation of the honey solution, and (3) a container which will be impervious to egg deposition.

Pharmacists are known to add parabens solution to certain preparations to inhibit mold and fermentation. A stock solution of this material is composed of:

methyl paraben, USP ..... 1.0 gm.
propyl paraben, USP ..... 1.5 gm.
and water to make 1000 ml.

Taking this lead, we diluted 25 ml. of the stock paraben solution with 75 ml. water. Ten ml. of this was then replaced with an equal amount of honey to make a 10 percent honey-paraben-water solution. Tests over a considerable period of time indicate that the addition of paraben to the honey-water does not repel the mosquitoes. It is nontoxic. Now we can prepare in advance a quantity of the honey-paraben-water solution and keep it indefinitely in the refrigerator. The material when added to a cotton pad, wick, or the new type feeder, does not mold or ferment.

Encouraging results were noted in preliminary tests using parabens-treated egg deposition papers. However, more extensive tests indicate that these chemicals crystallize out on the papers where they are then repellent to the adult female and also exert an ovicidal action on the eggs.

The problems relating to evaporation and egg deposition were answered by finely perforating, in a ½-inch-wide band, the sides of a 3-inch-high, 10 dram plastic capsule vial (Fig. 1). These perforations were made by punching the vial with a hot dissecting needle which created a roughened surface to which the adults can easily cling when feeding. A small roll of blotting paper is inserted to act as a wick and the vial is filled up to the perforations with honey-paraben-water solution. The tight fitting plastic cap through which an insect pin is inserted and bent over in hook fashion is then replaced on the vial.

This new type feeding device can be hung from the top of any cage being used for the adults. Eggs are not deposited on it and it is not necessary to refill the containers more than once every 2 or 3 weeks.

Attempting to establish colonies of mosquitoes for resistance studies from small numbers (approximately 10 to 15) of larvae or adults taken in the field often is difficult or impossible when the adults are released in an 18 x 18 x 18 inch cage. Whether this failure is due to unfavorable

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air currents, the vastness of the cage in comparison with the relatively small number of mosquitoes or some other factor, is not known. Believing the large size of the cage to be a detrimental factor, we reduced the sizes of our cages and struck upon the use of the following sleeve-type cage (Fig. 2) which is made of ½-inch mesh hardware cloth and tubular gauze bandage. The ½-inch hardware cloth is cut and rolled so as to make an open cylinder 1 foot long by 6½ to 7 inches

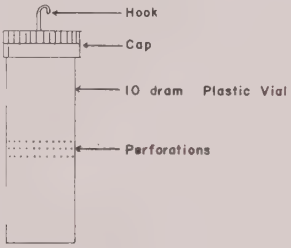

Fig. 1— SOLUTION FEEDER for ADULT MOSQUITOES

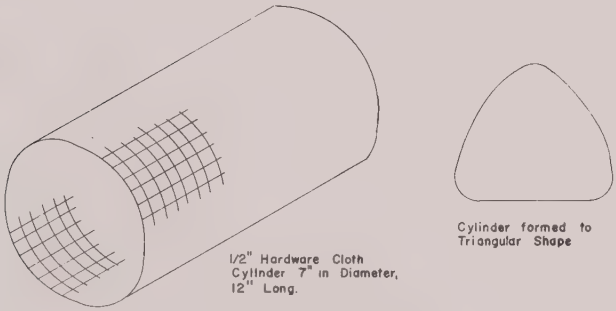

Fig. 2— SLEEVE CAGE

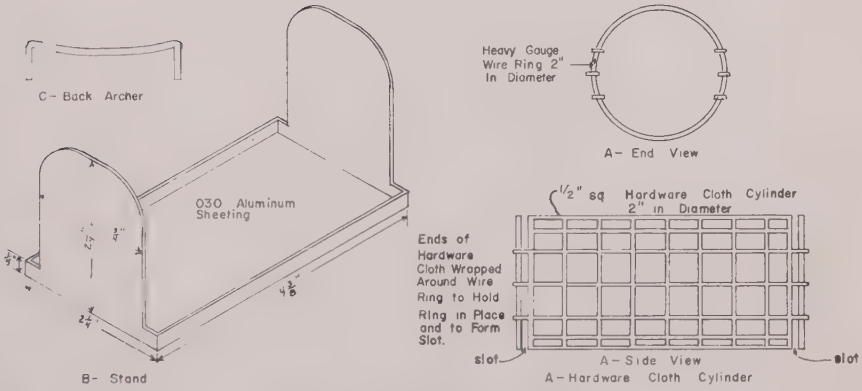

Fig 3— HAMSTER RETAINER

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in diameter. By bending the cut end wires alternately one way and the other it is possible to fasten the cylinder without need of solder. The rough cut ends of the cylinder can be covered with masking tape to prevent tearing of the 2-inch-wide tubular gauze bandage which is now slipped over the hardware cloth. Enough gauze is allowed over each end to permit closing the cylinder with a rubber band and/or a snap clothes pin. A cage of this type is smaller, is cheaply and quickly constructed and can be easily cleaned and decontaminated if necessary.

The compactness of the cage is an added feature. With slight pressure one can bend the shape of the cage—making it more or less triangular in cross-section. In such fashion there is no rolling from side to side and the cages can be easily stacked two or three high if needed.

With a large number of these small cages it was soon apparent to us that rabbits provide a rather inconvenient and time-consuming means of providing the small numbers of adults per cage with a blood meal. To eliminate this problem we shaved the backs of hamsters and found that the *aegypti* females were readily attracted to them. A small retaining device for the hamsters was made (Fig. 3) in which the animals can be easily placed in each mosquito cage for the feeding period. This device consists of three parts: the hardware cloth cylinder and two end rings (A). These rings are fastened in such a fashion as to form slots into which the end pieces of the stand (B) will slide. A short length of wire (C) is slipped under the caged hamster to arch its back and make it more vulnerable to the mosquitoes and also prevent excessive movement.

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VARIATION IN THE STRUCTURES OF HAIRS FOUND IN ANOPHELINE LARVAE FROM EAST PAKISTAN

S. R. HAIDER *

Some remarkable diversities in the structures of larval hairs were observed by the author during routine examinations of larval collections made available from different parts of East Pakistan. In routine examination of thousands of anophelines for species identification, those with variation in structures of their hairs were kept separate for detailed study. All the larvae under observation appeared in the 4th instar. They were mounted in chloral gum and then examined under a compound microscope.

The variations observed in the specimens are described below in detail.

Anopheles annularis Van Der Wulp 1884. (a) In one of the specimens of *A. annularis* variation was noticed in the posterior clypeal hair which was trifid at the right side while at the left it was simple (Fig. 1).

(b) In another specimen of *A. annularis* variation was again noticed, but this time it was in the sutural hair. A pair of inner sutural hairs were placed quite approximately at the right side while only a single unbranched hair was found at the left (Fig. 2).

Anopheles pallidus Theo, 1901. Variation in the character of larval hair was observed in one of the specimens of *A. pallidus*. The posterior clypeal hair had seven branches and not 2 to 5 as is usually present in *A. pallidus*. The presence of such character sometimes causes confusion and leads to incorrect identification. Puri (1954) in his identification table for anopheline larvae has also pointed out the large range of variation in the number of branches of posterior clypeal hairs in case of *A. pallidus* which sometimes creates difficulties in their correct identification.

The filaments of abdominal palmate hairs were, however, more than half as long as blades of leaflets. (Figs. 3 and 4).

Anopheles subpictus Grassi. Theo, 1902. Variation in the character of post spiracular hair was observed in a single specimen of *A. subpictus* larvae. The post spiracular hair at the right side had seven branches while the hair at the left consisted of four branches as usually found in case of *A. subpictus*. This is shown in figure 5. This naturally may cause confusion because post spiracular hairs with seven or eight branches are found in *A. sundaicus* which is a vector species in East Pakistan.

Variations in the larval chaetotaxy of *Anopheles* mosquitoes have been reported by previous workers in other countries too. Yates (1943), during the study of the 4th instar larvae of *Culex tarsalis*,

* Malaria Institute of Pakistan, DACCA/East Pakistan.

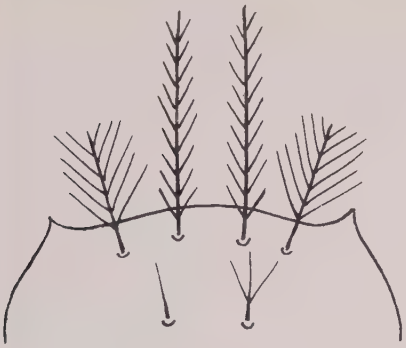

FIG. 1

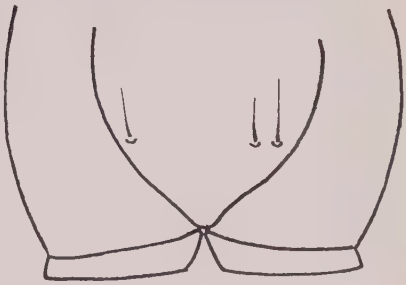

FIG. 2

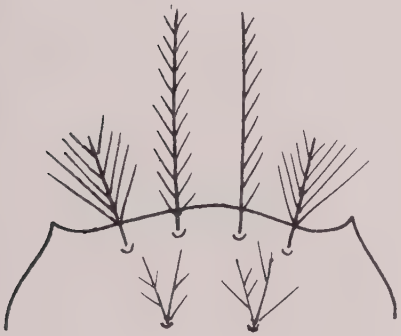

FIG. 3

FIG. 4

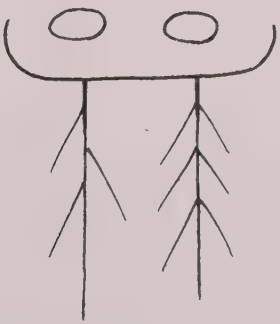

FIG. 5

observed considerable variations in several of their features.

Burton (1953) observed variation in the inner clypeal hair of *Anopheles quadrimaculatus* larvae. An abnormal specimen was found having three inner clypeal hairs instead of two. Mosquito larvae with abnormal gills, siphon and siphonal hairs were also reported by him previously.

Barr (1954) during identification of *Aedes vexans* larvae from Minnesota noticed a great deal of variability in the branching of the upper and lower head hairs.

White (1956) also observed variation in the number of branches in the head hairs. Fourth instar larvae collected from the field were examined for structural variation and compared with larvae reared in the laboratory.

According to Barr (1954) the amount of branching may not be rigidly determined genetically but environmental factors such as temperature may alter the expression of such a genetic trait.

But it was observed during the present investigation that the frequency of occurrence of variations in the larval chaetotaxy in the mosquito population was very low. Only four specimens of *Anopheles* mosquitoes were found with variations in their larval hairs out of thousands examined. The characters of larval chaetotaxy in *Anopheles* mosquitoes therefore appear to be quite normal in nature, and isolated samples which may exhibit variations are not statistically significant. Moreover these larvae were collected from all over the Province of East Pakistan from different types of breeding places.

It is therefore considered that the variations occurring in case of some of the larvae in the mosquito population may be just a freak of nature.

It is not possible to establish any correlation between the presence of variations in the larval chaetotaxy and any change in the environmental conditions such as temperature and types of breeding places.

Before any definite conclusion is made on enenvironmental factors, experimental work will have to be done. The experiments should be done under different constant temperatures in the laboratory, with different types and quantities of food supplied to the growing larvae to observe their effect on variations in larval chaetotaxy. Comparison should also be made between the larvae bred in the laboratory and those occurring in nature.

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** This was not seen in original.

FURTHER RECORDS OF MERMITHID PARASITES OF MOSQUITO LARVAE *

MARION E. SMITH
University of Massachusetts, Amherst

The occurrence of mermithid nematode parasites in mosquito larvae was reviewed in 1954 by Jenkins and West, who reported finding high infestation by the worms (subsequently described as *Hydromermis churchillensis* by Welch, 1960a) in larvae of *Aedes communis* (DeG.) in northern Canada, with light incidence in two other species of *Aedes*. In 1956 Laird reviewed world records, and later Welch (1960b) summarized mermithid parasitism in North American species of mosquitoes. In 1953 and 1955 Frohne also reported the presence of mermithids in *Aedes* larvae in Alaska, with heavy infestations in *A. communis*, and added several species to the list of known mosquito hosts given by the preceding authors.

In 1959 two instances of parasitism were observed by the author in *Aedes* larvae collected in the vicinity of the Rocky Mountain Biological Laboratory in Gothic, Colorado, at elevations of about 10,000 feet.

On July 3, a last instar larva of *Aedes pullatus* (Coq.), collected at the Gothic Picnic Area of the Gunnison National Forest, was observed exhibiting abnormal body thrashings. Upon examining the specimen, a mermithid worm was seen in the process of emerging from a large hole on the side of the thorax of the larva; a second one followed shortly. The two parasites differed markedly in length and diameter, the first being about 17 mm. long and the second one about 7.5 mm. Parasitism of this species of mosquito by mermithids had not previously been reported.

On July 15, 138 living nematodes, along with many dead culicid larvae, were taken from the bottom of a rearing jar which had contained larvae of *A. pullatus* and *A. communis* collected earlier in Elko Park (White River National Forest). Many of these parasites were larger than those found emerging from the *pullatus* larva, some attaining a length of about 22 mm. It was not determined whether only one or both of the species had served as host.

Dr. H. E. Welch, to whom the specimens were sent for examination, reported that the parasites were larval mermithids, which lack characters for

* Entomological contribution number 1346 from the Department of Entomology and Plant Pathology, University of Massachusetts.

positive identification, but were close to or identical with larval specimens of *Hydromermis churchillensis* Welch, a mermithid reported from several species and genera of mosquitoes, including *Aedes communis*. Only one species of parasite was represented in the Colorado material.

In his discussion of the life cycle and bionomics of *H. churchillensis,* Welch (1960a) pointed out that parasitized mosquito larvae "are arrested in their development and fail to pupate, a condition which results in the accumulation of parasitized larvae in the pools" and tends to produce artificially high rates of parasitism. The Colorado specimens bear out this statement, for the parasites were discovered at the end of the breeding season, after all healthy larvae in the culture and in the original places of collection had completed their development.

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The Annual Meeting of the **NEW JERSEY MOSQUITO EXTERMINATION ASSOCIATION** will be held at the Hotel Haddon Hall, Atlantic City on March 21st, 22d and 23d, 1962. The four-session program will include reports and exhibits of scientific and popular interest pertaining to mosquitoes and their control. Along with the announcement of this meeting came the information that the color film "The New Jersey Mosquito Story" is now available through the New Jersey Association at $225.00 a copy.

OPERATIONAL NOTES

P. BRUCE BROCKWAY, JR.

During the past few months there has seemed to be a gathering interest in the Operational Notes and we are happy that some of our authors have been sending in notes on equipment and other operational data that are very useful in mosquito control activities.

Bob Armstrong writes us of his use of a booster pump used in conjunction with his DYNAFOG SR. He writes as follows:

"We have recently been using a JABSCO 'Minnie Puppy' pump to supply insecticide to the DYNAFOG SR. fog generator. This is a d.c. battery-operated pump designed as a bilge pump for small motor boats. Operated from the battery of the fogging truck it pumps insecticide from two 55 gallon barrels and has more than enough pressure and volume for the DYNAFOG SR. A bypass valve returns the excess flow of insecticide to the barrels.

"The DYNAFOG SR. seems to produce a better and 'heavier' fog at 45 g.p.h. than it did at 20 to 30 g.p.h. judging from field observations. Four gallons of 90% malathion miscible are diluted with 100 gallons of fuel oil to make the fogging solution.

"The 'Minnie Puppy' pump has a neoprene impellor that swells after several hours of use. We take this impellor out after the evening's fogging operation and let it dry for a day or more. Another impellor is used the following night. The inside pump shaft gasket also swells and bulges out of place. It has to be replaced occasionally.

"We consider the pump arrangement a makeshift affair and would like to have suggestions from the experiences of other mosquito control workers. We would also like to know if others have increased the DYNAFOG output to 45–50 g.p.h. and find the fog more effective at that increased rate."

Then, going to the other side of our country—Ted Raley and his associates have perfected a granule "shooter" that has proved most useful in his district for applying granule insecticides. This little "shooter" could be most useful in most any mosquito control district. Ted's write-up appears elsewhere in this Section.

Returning to the East coast area, Russell Gies, of Media, Delaware County, Pennsylvania, writes that in eastern Pennsylvania there is a trend toward more insecticidal fogging for whole towns as people generally see a measure of improvement of results where fogging is done. Furthermore, Russ is encountering the same problem as many of us who have districts where suburbs are expanding and new homes are constantly under construction. He states, "In rapidly growing suburbs construction of sewers is lagging behind, so more mosquito breeding is taking place as a result of sewage pollution." We all have this same problem to one degree or another. Perhaps the reader would have a suggestion for Russ.

It is also interesting to know that Russ was able to apply his DDT "Attaclay" at a cost of $1.96 per acre. This includes the entire cost of not only the material but all related expenses. How does this cost compare with other Districts? Russ has used a fixed wing airplane for treating this same area for 16 consecutive years with ½ pound per acre per application and there have been 3 or 4 treatments each year. He notes that in spite of this amount of chemical insecticide, wildlife has increased and the conservation officials in a wildlife refuge told him that now they have five times as many ducks and songbirds as compared to some years ago, and that wading birds, fish, frogs, and other such animals have also increased. Resistance studies through the use of W.H.O. test kits do not indicate any DDT resistance in the *Culex pipiens* or *Aedes vexans*.

The Delaware County Mosquito Control Commission has had "Ten Commandments for Mosquito Control" printed and the Commandments are distributed by the local Boy Scouts. These "Ten Commandments" should be followed in any mosquito control district. Although many of our readers may know them or their counterpart by heart, they are reprinted below for good measure:

1. Eliminate all standing water around your premises to prevent mosquito breeding.
2. Flatten all tin cans or puncture both ends and dispose of any water holding receptacles such as old paint cans, old tires, etc.; wherever water may collect.
3. Drain clogged roof gutters and flat roofs.
4. Seal cesspool covers and screen vents.
5. Change water twice a week in troughs, bird baths, fountains, etc.
6. Be sure that screens fit tightly, that doors open out and that screen mesh is not larger than 16 to the inch.
7. In rural areas cover all standing receptacles such as rainbarrels with tight netting.
8. Empty the watering can, the wheelbarrow, etc. after using around the gardens.
9. Treat foundation or other water holding excavations, wheel tracks, depressions caused by improper grading and small swampy areas with kerosene oil or fuel oil at weekly intervals. Spray screens and around door and window frames with a household DDT spray 2 or 3 times during the summer to help keep mosquitoes out. Mosquitoes that do find their

way indoors may be killed by using an aerosol insecticide "bomb."

10. Cut down tall grasses, weeds and other excess vegetation that harbors mosquitoes during the day on your own or on adjacent property.

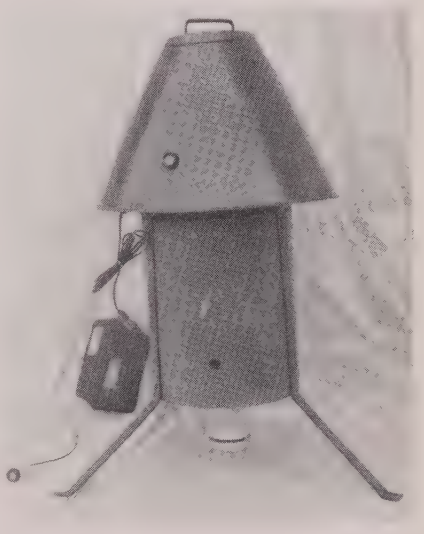
Many of the mosquito control districts and many of the state organizations have been making an intensive study of personnel and employment problems as well as establishing one form or another of job classification. This is one field that was neglected during the early years of mosquito control in some areas. However, during recent years while we are trying to maintain a respect for individual accomplishments and reward the conscientious worker, some mosquito control organizations have made conscientious efforts not only toward protecting their employees, but also giving them a specified plan to follow in order to get a higher job classification. Some districts are under Civil Service regulations, others are not, but usually some reference is made to Civil Service regulations. The San Joaquin Mosquito Abatement District of Stockton, California, under the management of Mr. Brumbaugh, has made some commendable steps in the field of employee relationships, and I am sure that their employees are rewarding the Abatement District by giving better service to their governmental agency. First of all, the District has recognized problems of employees and has recognized the need for ratings. Furthermore, each employee is given recognition for his performance of his duties. Diplomacy and understanding are most necessary. They have developed a fair and unbiased rating program and although they admit that there are some difficulties that have arisen from this rating program, they are gradually being overcome. Employees have different attitudes, feelings, and behaviors and these are respected. High employee productivity is closely related to high employee morale. All these suggestions and discussions of their performance are very well described in an article written by Mr. Brumbaugh under the heading of "A Constructive Approach To Performance Rating." Those districts which are studying employee problems should contact the San Joaquin Mosquito Abatement District for their suggestions and information.

Examinations for field employees have always been a problem to mosquito abatement districts because each district faces different problems not only in terrain but most certainly in the species of mosquitoes and their habits. San Joaquin has a good example of such an examination for their inspectors.

A copy of their October, 1959 Classification Manual would be most useful to districts facing the problem of employee classification responsibilities and rights.

Electric Eye Switch for Light Traps

It has always annoyed me when I have found out that one or more of our light traps had been out of order for the night because a householder had turned off the switch for the power to the trap or perhaps the area had a power failure for a few hours which in turn caused the light trap to turn on at the wrong time of the night. There are also problems concerning the light trap clock itself that would confuse trap counts and samples. These problems, plus many others, caused the writer to investigate the possibility of using an electric eye for turning the trap on and off automatically. We were, indeed, very fortunate to find that one of the leading electric eye manufacturers was located locally and Mr. Don Sell of the Don-Ell Corporation gave us suggestions and cooperation which ultimately led to a very satisfactory electric eye switch for our light traps.



Light trap with electric eye mounted on bonnet of trap. Lower left hand corner is the electric eye and above it the old-fashioned clock.

These switches are small, and according to all the tests that we have made they are foolproof; furthermore, they are easy to install on the standard light traps, such as those manufactured by the Concession Supply Company of Toledo. Test switches and power outlets can easily be spliced into the wiring system if desired.

This device is the answer to the light switch problem of a district that desires mosquito samples collected during early evening, night, and early morning hours. The switch is sensitive to the lack of light and is activated to turn the trap on as the sun goes down. During the month of June, the average time of light trap "on" period was from about 8:15 p.m. to 5 a.m. It is recommended that the eye be pointed toward the east and that it be mounted on the top of the bonnet of the trap rather than on the side of the unit.

Clocks require electricity to operate, but this unit does not require this source of energy except during the twilight or dark hours. Maintenance and service to the clock unit is eliminated.

We were very pleased to realize that the cost of the electric eye switch was about one-third of that on an electric clock, and it is much easier to install on a light trap. Presently, the Toledo Area Sanitary District is using six of these units and we are well satisfied with the service that they are giving us.—P. B. B., Jr.

VIBRATION-PROOF MOSQUITO TRAP

A. H. CAMP

Operations Manager, Lake County Mosquito Abatement District, Lakeport, California

For several years we have been operating mosquito light traps in cooperation with the Bureau of Vector Control, Department of Public Health in California. These are "American Model" traps. Even using "Vibration Service" light bulbs, these traps take considerable bulb replacement.

Last fall we decided the bulb failure was due to vibration from the fan motor, so we tried to find a way to eliminate it. We finally came up with the idea of changing the solid motor mounts to springs, using three quite stiff coil springs fastened to the tube sides, suspending the motor in the center of the tube. We feel this has been very satisfactory. Normally we would have replaced four to six bulbs by this time of year and this year we have replaced only two.

A GRANULE "SHOOTER"

T. G. RALEY

Manager, Consolidated Mosquito Abatement District, Selma, California

Applying the new hard core insecticide granules by a controlled air flow intrigued Lee Crawford, Foreman, Consolidated Mosquito Abatement District in the fall of 1960. Faced with the task of larviciding miles of weed-grown drain ditches, cattail-filled ponds and other large mosquito sources where liquid sprays had always given uncertain results, he started searching for something better and faster than the HORN hand applicator to apply granules. Power spreaders mounted on the vehicle were excellent for open areas with low vegetation but this type of mosquito source was only a small fraction of the total problem. Something that would shoot the granules over high vegetation, fences, ditch banks and other obstructions in a directed pattern seemed the best approach, so Lee started experimenting.

Each District Jeep is equipped with a constant pressure spray unit, supplied by a heavy duty air compressor operated from a power take-off on the vehicle. After much trial and error an airgun (Fig. 1) was developed that served the need well. An operator sitting in the Jeep (Fig. 2) could shoot the granules 35 to 40 feet in any direction desired. Shooting into the wind shortened the throw somewhat but rarely required moving to rougher ground to treat down wind, or stopped the operation. Blow-back and poor aiming has occurred but this presented no hazard from the hard-surfaced, dust-free granules. Larval kills in even the heaviest vegetation are excellent.

FIG. 1.—Consolidated Mosquito Abatement District's "home-made" airgun.

FIG. 2.—Operator applying granules with the airgun in comparative comfort.

The application of granules with an airgun has very quickly developed into a welcome aid in aquatic larvae control. Commercial sand blasting equipment to replace the home-made guns and insecticide can now be supplied at reasonable rates. Equipment costs will probably determine whether the inexpensive district-made gun or the finely tooled commercial gun is used. Either will do a good job of getting the granules over and into areas covered with vegetation.

EDITORIALS

WHAT'S IN A NAME

I am in favor of short, distinctive names for scientific journals. MOSQUITO NEWS is unique among appellations of society publications. The fact that it has retained the name for more than two decades deserves attention. "Journal of the American Mosquito Control Association" is a long term which, when referenced, would need to be reversed as "Amer. Mosquito Cont. Assoc. J." One unfortunate thing is that a name change results in the journal starting with volume 1 and a new place on the library shelves. If our members feel we need a more sophisticated name, I would vote for "Culicidology Journal." This pins down the subject just as "Journal of Parasitology" or Journal of Helminthology." If we abbreviate the name of "J. of Amer. Mosquito Cont. Assoc." to JAMCA it could well be confused with JAMA (J. Amer. Med. Assoc.). Furthermore, under the guise of "J. Amer. Mosquito Cont. Assoc.," we might be setting the stage for articles on everything from house flies to rats. MOSQUITO NEWS is a good name and the front cover plainly tells its readers that it is the journal of our organization.—HELEN SOLLERS.

There is an old saying that "sticks and stones can break my bones, but words can never hurt me." Although recognizing this homily to be basically true, there are few of us with the equanimity required to bear up under names that we dislike. This is proving true for many in connection with the name of our American Mosquito Control Association publication, *Mosquito News*. This compact, prim little quarterly has through the years almost without notice metamorphosed from a news letter (described by one of our members as originally a "mimeographed poop sheet") to a first-class scientific journal, read and respected throughout the world. Because of this transition into a top-flight journal the title of "Mosquito News" has in the minds of some of our members come to seem far too unsophisticated for such an erudite proceedings as our publication has become.

Because of this concern, it is believed to be appropriate to editorialize briefly on the subject. Perhaps by so doing, the membership may be persuaded to do some serious thinking on the subject and to be armed accordingly with definite ideas well before someone decides to make an official attempt to change the name.

Changing the name of any established journal is a very serious matter as it will leave a wake of misunderstanding and error among librarians and literature searchers long after the change itself has steamed away over the horizon. That the storm can be successfully weathered, there is no doubt, but will the chaos and clamor be worth it all?

Some say "yes" because they feel that a marked increase in prestige will result. These individuals say that a research director is not very impressed when asked to support publication or reprint costs for an article to be published in some sort of a "mosquito newspaper"; whereas, he wouldn't swallow twice when asked to pay such costs in connection with a publication augustly entitled "The Journal of the American Mosquito Control Association."

The English are strong on traditions and many old customs are preserved by them long after they have become anachronisms simply because of this racial characteristic. Many members of the American Mosquito Control Association probably regard the name *Mosquito News* and its preservation in a similar light. I for one concede this to be a perfectly valid reason for retaining the name. Nonetheless, I favor changing *Mosquito News* to *Journal of the American Mosquito Control Association* because such a change brings full

recognition of the stature which our peppy little journal has attained.

The Committee on Publication of Mosquito News has been asked to consider this problem and to make an appropriate recommendation to the Board of Directors to guide them at their 1962 meeting. Believing this to be a problem for membership voting only after an educated public opinion has been formed, the subject is here being opened for discussion and debate. Between now and next March, all members feeling strongly enough one way or another on this problem to write letters, are urged to do so, addressing them to our president, Dr. W. E. Bickley. Perhaps by so doing, the Board of Directors can gain some insight as to whether or not the majority of the membership desires such a change at this time.—KENNETH L. KNIGHT, Chairman, Committee on Publication of Mosquito News.

. . . The AMCA meeting at Anaheim decided that the name *should* be changed, to "Mosquito News . . . The Journal of the American Mosquito Control Association" I don't remember that it was left in doubt. There was a suggestion that after we saw what membership thought of the above, there could be further consideration given to dropping the "Mosquito News" bit but it seemed to me that all, even those who wanted the "Journal" part added, felt that MN is now established and should not be changed. The original objection to MN as "undignified" has been pretty well outdated, it seems to me . . . —A. W. MORRILL, JR.

Libraries are bulging with Journals of the American Association of Such and Such. Each society must have its JOURNAL. A new journal is advertised every time the junk mail is delivered. Unlike ordinary scientific societies the American Mosquito Control Association has a journal with a unique, glamorous, distinctive, meaningful name, MOSQUITO NEWS! News about mosquitoes. Unsophisticated? Perhaps, but is sophistication more desirable than individuality? Do we need to create an impression with a more sophisticated name? Will a new name help us attain our objectives? Will we reach more people, or will the Journal of the AMCA be buried among the 1001 other journals. Is non-conformity always bad?—WILLIAM E. BICKLEY.

CONTRIBUTORS TO THE AMCA MEETING IN GALVESTON are reminded that projection of slides will be made in a large room. For use as slides, tables should be organized on the scale of the well-known "Radio Mats," with no more lines and columns than can be accommodated at that size type in an equal space. Charts and graphs also should be designed with the same limitations in mind. **IT IS BETTER TO HAVE TWO SLIDES THAT CAN BE SEEN THAN ONE SLIDE THAT CANNOT BE READ.**

NEWS AND NOTES

AUSTIN W. MORRILL, Jr.

No Doubt You Have All Received the Proceedings of the Eighteenth Annual Meeting, and we'd like to call your attention once more to the masterful and timely address given at these meetings by Assemblyman Carley V. Porter, which is printed there, starting on page 13. We thought this was a most worthwhile summation of our problems at the time we heard it and in re-reading it now we still think so. It isn't every "layman" who is this aware of the deeper aspects of mosquito control and Mr. Porter's understanding is not only warmly satisfying to us mis-understood types but gives us a good bit of ammunition for using with our own legislative and administrative hierarchies whom we, from time to time, feel the need to educate.

The Need to Educate the Public and Particularly the Newspapers is fully evident to us all, judging from the number of clippings we have received from all over the country, in which "Do Insecticides Cause Insanity?," "Insecticide Death Ruled Manslaughter," "Insecticides Linked to Mental Ills" and the like were the predominant headings. In the mental health bit, the symptom of disorientation which is considered an early warning of many kinds of poisoning, is used as a springboard by which insanity is linked to "insecticide" and then, soaring off into the wild blue yonder by means of the statement that "organic phosphates" are considered by many to be "less toxic than DDT," we have a wonderful frolic again in the fields of fantasy where in past years national magazines have sought to frighten America into repudiating or forbidding any sort of chemical insect control. Many of us spend considerable time spreading the True Word about all this. Virginia, for instance, as reported in VMCA's *Skeeter,* this summer had a two-part radio program in which one of the superinten*dents,* Jack E. Dent, that is, answered questions on mosquito control for some fifteen minues. Jack does this two or three times a year and has for several years. He feels, with justification, that this has been an important aid, for there has been a steady decrease in the number of households found breeding mosquitoes and a corresponding rise in public support of their program.

In other areas, Chet Robinson had yet another fine write-up in the *Oakland Tribune,* while George Thompson reported that his public was showing an increasing interest in the program and that part of this program was the policy of giving phone-callers full and informative discussions of mosquito control, a policy which was beginning to pay off.

Speaking of George, way back in June he reported something which we meant to bring to your attention but somehow managed to leave out of the last issue. A badly overloaded Imhoff tank, long a source of *Culex quinquefasciatus,* was treated with Paris green and vermiculite pellets and good control was obtained for *eight days* thereafter. Treatments in polluted city ditches met with equal success.

Dr. Fred Bishopp was recently appointed by Governor Bryant of Florida to the Lee County (Florida) Mosquito and Hyacinth Control Boards. We wish we could reproduce in full the lengthy encomiums which the papers of Florida ran on Dr. Fred and his productive career, but you all know him and have more than once joined in the plaudits for our AMCA's ex-President. The combination of water hyacinth and mosquito control into one organization is a natural development and has been placed in the capable hands of Wayne Miller. At a recent meeting, Wayne was elected president of a newly formed Hyacinth Control Society, with William Dryden as editor and Thomas Fultz one of the Directors.

Meanwhile, According to Uncle Harry Stage, the State of Washington mosquito control program was approved by the Legislature and is now legal. And operating. Thus getting off the ground the program stirred up by Ye Olde Harry while he was reposing among them and before he "retired" to Oregon. That reminds us, it's been suspiciously quiet up there in Oregon lately. What're you up to, Harry? (See what the children are doing and tell them to stop it.)

Helen Louise Durkee wrote us, just before taking off for a trip up the Inland Passage and into the Yukon Territory. We hear the mosquitoes are just amazing up there, and the scenery is too, so we know that they both had a fine time with net and camera. Anyway, Helen wrote that she'd been appearing lately before classes to carry the word in connection with the Contra Costa (Calif.) Mosquito Abatement District's program. So maybe we're making progress after all, with all these good workers working. How about you, you other people whom we don't hear from? Drop us a card, do!

Helen Louise Also Sent Us a Letter From Another Helen . . . Helen Sollers . . . Who Has Been Traipsing About All Over. First she went to Jamaica and attended a few sessions of the Malaria Training Center, and floated down the

Rio Grande on a bamboo raft and had an elegant time. Then home to Washington and a flying start to Cape Cod for another week. But not of floating around on bamboo rafts. Though nice in its restrained New England way.

THE UNIVERSITY OF CALIFORNIA AT DAVIS HAS COME UP WITH SOME MORE FACTS and figures which might be of interest to many, we think. F'rinstance . . . 12,000 mosquitoes were marked and studied for flight patterns. It was found that the females flew upwind, as previously suspected, in search of blood meals. Thirty-five per cent of the *Culex tarsalis* females could lay viable eggs without a blood meal but this autogeny was paid for in reduced flight range (about 1¼ miles) and fewer eggs. Normal flight was from 8 to 12 p.m. (or do we mean 8 p.m. to 12 m. . . . well, anyway 2000 to 2400 hours) and temperature was an apparently deciding factor. Rice fields were found to be ideal *Culex* and *Anopheles* breeding sites. (This is *news?* Well, it's a note.) This all came out at a recent meeting of the Mare Island Area Mosquito Abatement Committee, which is a committee of managers of mosquito abatement districts of the northern San Francisco Bay Area and representatives of the various Army, Air Force and Navy installations thereabouts. The committee started at Mare Island, once infamous for its villanous mosquitoes, and it still meets there, attended by such AMCA'ers as G. PAUL JONES, of the Marin Co. (Calif.) MAD, EMBREE METZGER, of Solano County MAD, BILL RUSCONI, of Napa County, TOM LAURET, of San Mateo County, and BASIL MARKOS, of the California State Bureau of Vector Control. These worthy gents not only give the military installations unlimited assistance, to the benefit of all Americans, but they have been finding value in these occasional get-togethers as a means of coördinating their own programs; needless to say, the programs are all in fine shape!

THE LOUISIANA MOSQUITO CONTROL ASSOCIATION has just issued a report which contains some very interesting and heartening information. Their first construction of access ditches into salt marsh areas, to reduce breeding of *A. sollicitans* has been initiated in St. Mary Parish, where the applicability of this time-tested method to Louisiana conditions will be tested or demonstrated. This construction was the culmination of eight months of intensive investigations on types of breeding areas and suitability of various control methods to each. Four other pilot projects showing the applicability of other methods of control are planned for early commencement as well as expansion and intensification of surveys and preliminary tests. Professor ED HATHAWAY, the Executive Director of the Association, sums it all up by quoting an early investigator who counseled, "Keep the dry lands dry and the wet lands wet."

ANOTHER NOTABLE MEETING WAS THE ALL-SERVICES CONFERENCE OF MILITARY ENTOMOLOGISTS held in Washington 23 through 27 October. In this group are no less than *sixty-eight* AMCA-'ers, so we obviously can't list them, but we think this is pretty good representation, considering that most of the non-AMCA'ers do not have any connection with mosquito control. (We aren't proud of several notable omissions and shall work to correct *this*!) The military types had a very fine meeting we hear (we weren't there) and were further enlightened by other AMCA'ers, including DICK FAY, of the U.S.P.H.S., JOHNNY FALES and ART LINDQUIST of the U.S.D.A. and ROY FRITZ of ICA. They almost had a quorum, if BILL BICKLEY had thought to call it that; might have had some mighty fancy legislation for AMCA!

ALTHOUGH ED SMITH'S NAME NO LONGER APPEARS ON OUR MEMBERSHIP LIST (this *must* be some sort of *mistake!*) a report sent out by ICA concerning his work in Thailand will be of some interest to a number of our members who have been or still are intimately concerned with that country. Ed has been working with the Thai government for the past nine months to bring the malaria control program into line with present needs and to incorporate new medical advances, and his role has been an important one, according to ICA. We're not surprised to learn that Ed has been doing a fine job, and we are glad to learn that about one-fourth of the Thai population now lives in malaria-free areas. Since the U.S.-sponsored road building program has now opened up many formerly inaccessible spots, concentration of efforts is now on the eradication of malaria in these rural areas. Improved roads have not entirely eliminated the effects of climate and geography. On one trip out from Maesarieng, Ed is said to have taken but an hour for the 40 mile trip, while the return trip, made during a monsoon rain, required five hours, spent in chopping two huge trees out of the road, where the winds had felled them. Ed has been with ICA for five and a half years now, his former assignment having been in Indonesia, and on a recent trip back here he indicated that his enthusiasm for the fur places is still undimmed.

GEORGE BURTON, WHO SPECIALIZES IN FUR PLACES ABOUT AS SUCCESSFULLY AS ANYONE is now in Georgetown, British Guiana, and we thought we might wait to give the latest word on him until he had given us the latest word on the recent political events down there. Our latest letter from George said that they are finding that their strain of *Wuchereria bancrofti* is behaving in a highly individualistic manner, though repeated tests have checked, one with the next. This dulls the edge of monotony quite successfully, we imagine. Contrary to general impression, George says the climate is fairly pleasant. A voluminous report from his station gives data to show that chemotherapy is being surprisingly effective, at least as a suppressive, something which is distinctly news to tyros like us.

RUSSELL FONTAINE RECENTLY VISITED THESE SHORES ON A BRIEF BUSINESS CUM VACATION TRIP, arriving via Central America. He is stationed in Lima, Peru, one of those entrancing cities, and enthusiastic about the future of both inter-American relations and inter-American mosquito control.

STAN CARPENTER IS NOW BUSILY SUMMARIZING the results of his first two years' work with the California State Bureau of Vector Control and, as we have indicated previously, he has a lot of good observational data to clinch what has previously been conjecture and to light the path toward further knowledge and even control of our vacationland species. The results of his summaries will appear as a series of articles in the BVC's *Vector Views,* which is distributed worldwide and will be distributed to you, too, (if you don't already get it) if you will write in and ask for it. Address: California State Department of Public Health, 2151 Berkeley Way, Berkeley, California.

MAYBE WE SHOULDN'T HAVE STARTED THIS. There've been an awful lot of folks moving around lately. You may have noticed some changes had been made, in the list that was published in the last issue. But even some of *those* have changed. MAJOR BOB ALTMAN, for instance, has left the University of Maryland to be Liaison Officer of the Armed Forces Pest Control Board at the Orlando (Florida) laboratory of the U.S. Department of Agriculture. Address: P.O. Box 3391, Orlando. MAJOR CARLYLE NIBLEY replaces Bob at the University of Maryland, coming back from a productive four years on Okinawa. MAYNARD JOHNSON is correctly listed in the September issue but it doesn't tell you that he has left ICA to settle down cosily with research at Ft. Detrick, Maryland. Or that WALTER BARRETT has left the Navy for the Wild Blue Yonder and is now with SAC Headquarters, at Offutt A.F.B., Nebraska.

BILL CANTELO has shaken the dust of Guam, and the Navy, from his feet and gone out to Thailand for ICA, address as shown in the list (still an APO from San Francisco and we didn't want you to feel confused). ADAM FULMER is at the same APO, in the Army Liaison, thus taking his malaria control abilities back to Southeast Asia where they got so innocently aroused years ago. What big changes in our lives little events may lead to! Well, out of the old revery, boy, and back to the listings. . . .

BILL KRUGER has left Columbus, Georgia, for a new address at 3709 Wilmore St., Dayton 16, Ohio, and DR. BILL GOODWIN has left Scottsville, Virginia, for USOM, Haiti, % American Embassy, Port au Prince, Haiti, both kind of biggish jumps, climatewise. (How about a word or two on exotic Haiti? Or even exotic Ohio??) H. C. (CHAPPY) CHAPMAN and JACK KELLER, both of whom were once at Orlando, blissfully mosquitoizing in lotusland, have also made biggish jumps, Chappie to Fresno, California, where he is located, as a coöperator, with the California State B.V.C., at 5545 Shields Avenue, and Jack to 1016 Beverly Dr., West Point, Mississippi. TAKASHI ISHII, taking an even bigger flier, hopped from Tohoku University at Sendai, Japan, a lovely city but far from tropical, to 318 Atherton House, 1810 University Ave., Honolulu 14, Hawaii. We think we told you DAVE REED has moved from Tulare MAD to Fresno-Westside, in Firebaugh, California, where his address is now 1421 R St.

MERLE L. KUNS went from the Georgia Department of Public Health to the Department of Veterinary Science of the University of Wisconsin, Madison 6, and CALVIN SPENCER, still with the USPHS, was transferred from New York to Love Field, Dallas, Texas, one of our favorite cities. (Those mosquitoes are probably coming from down in Turtle Creek, Calvin,—at least they used to when we were a boy.)

WELL, IF YOU WONDER ABOUT ANYONE ELSE, YOU CAN GET THE LATEST SCOOP FROM OUR EVER-FAITHFUL EXECUTIVE SECRETARY, TED RALEY but one thing you can't get from him in the way of changes to the list, since the people involved were too durned modest to tell him, is that there have been some changes in grade among the military. So you may like to know that TED BLAKESLEE is, and has been for some time, a Lt. Colonel, as has HAN SCHENKER. TEO SICAY, a Major, is now retired, and still in Savannah, Georgia. DICK HOLWAY is a Captain, USN, and SAM HILL, who is now a civilian with USDA, and JOE WEBB, who is NOT a civilian, being entomologist with the U.S. Army Surgeon General's Office, are both full, or "chicken" Colonels, we are glad to say. There've been some mighty fine promotions announced for the Navy recently and we are anxious to tell you about them, but will have to wait until the boards are actually on the respective shoulders, we guess. And you fellas who've had a change of status, like from Ensign to j.g. or Mr. to Dr. and like that, let us know it, will you? Doggonit, it's part of your *address!*

DICK FROLLI reports from his Kings Mosquito Abatement District (California) that measurements of *Aedes nigromaculis* in the western part of his district showed a 37-fold increase in resistance to ethyl parathion, even though the material has been used in that area for only two and one-half years.

WE CAN'T IMAGINE HOW IT HAS HAPPENED THAT WE HAVE NEVER BROUGHT YOU any information in our Who's Who on one of our oldest and best known members, but there it is. We

haven't. So now we want to make up for this sad deficiency. JOHNNY MULRENNAN was born in Bloomingdale, Florida, in 1906 and graduated in Entomology from the University in 1932. (Thank heaven for *American Men of Science,* which lists all these things!) Johnny's folks had then, and he still has, a farm, which in Florida in the Tampa area means citrus orchards; starting out at 160 acres, Johnny has built it up to 380 acres. After graduation, however, and despite a Depression some of you may remember, Johnny was offered a job with the Rockefeller Foundation with whom he stayed for several years. This was followed by a couple of years with the Texas State Board of Health in Austin, Texas, after which he returned to Florida and commenced his productive career with the Florida State Board of Health. He was in Pensacola for a year or so and then moved to Jacksonville, where he still is. Not long after his entry into the State Board of Health, Johnny made himself invaluable to the military not only by sharing with us his wide knowledge of the local mosquitoes and their odd habits, but also by assisting us in setting up control measures in the State which held so many military bases. One of Johnny's two sons, and our fellow AMCA'er, is Johnny Jr. (Lt. jg. USN), Assistant Officer in Charge of the U.S. Navy Disease Vector Control Center, Alameda, California. There are also two daughters and a passel of grandchildren. In all of this activity, Johnny has retained his interest in baseball, in which he is a real buff, and also in several social organizations. His scientific affiliations include, beside AMCA, the Entomological Society of America, the American Society of Professional Biologists, the Florida Entomological Society and the Florida Anti-Mosquito Association. He was vice-President of the National Malaria Society and has been President of both the Florida entomological societies listed above and of the Florida Public Health Association. And of course, as you know, he is the Chief Entomologist of the Florida State Board of Health and the person who established the remarkable research organization at Vero Beach.

JIM NELSON, whose correspondence course (via Purdue University) *some* AMCA'ers MUST be taking because there are 1,400 takers, is, of course, Editor of *Pest Control Magazine,* as he has been for its fifteen best years. He was born in Cleveland, Ohio, in 1920 and attended Hiram College, in that state, where he received his degree in economics. Quickly perceiving that Economics was Not Enough, he began taking short courses in pest control at Purdue, supplementing graduate studies at Western Reserve; during the war years he was a special agent in the Counter Intelligence Corps. Getting knowledge out of obscure situations being part and parcel of the surveillance required for good pest control, Jim ended the war already well-equipped to join our ranks, which he promptly did. In addition to collecting and disseminating information on pest control in urban and industrial areas (and articles on mosquito control by water management in not-so-urban areas), Jim has time to be a trustee of his Alma Mater, of the Calvary Presbyterian Church of Cleveland, and of the YMCA, and to pursue the hobbies of photography, music and travel. A well-rounded gent.

CARLYLE NIBLEY, JR., otherwise known as NIBS, one of the Army's more energetic mosquito controllers, is that *rara avis,* a Washingtonian. After graduation from Woodrow Wilson High School in Northwest Washington (D.C.), in 1942, where Nibs modestly and with characteristic self-denigration says he was in the top of the lower third of the class, he went immediately into the Army and fought his way up through the ranks and through the South Pacific. His malaria control unit was successively in Hawaii, the Solomons and the New Hebrides. Returning to Washington in 1946, he entered American University, where he received his B.S. in 1949 and then went back into the Army to be one of the officers in the new 37th Preventive Medicine Company which controlled mosquitoes for the Armed Forces in Korea, during that war. Following this, Nibs was at Walter Reed; Headquarters, Third Army at Atlanta, Georgia, where he did that interesting work on different color lights in mosquito light traps; Ft. Detrick and Okinawa, from which he has just returned, as noted previously. Major Nibley is presently attending the University of Maryland, where he says the entomology dished out by PRES BICKLEY is tough but going along well, but that the Russian is killing him. Sounds bad. Nibs, incidentally, got his M.S. from Maryland in 1957, so maybe Bick, being forewarned, got him to take Russian to work off some of that extra energy.

DISTINGUISHED SERVICE. We are happy to quote below from the USDA Employee News Bulletin for September 13, 1961, the notice of the presentation of the Department's *Distinguished Service Award* this year to Art Lindquist.

The citation of the award, presented in Washington, D. C., last May reads:

"For original research and forceful leadership in improving the health and welfare of man and livestock through the development of new methods for controlling insects of medical and veterinary importance."

A colleague has added that another way of stating it would be "For 30 years of inspiring leadership, hard work and unequalled productivity in the field of research on the control of insects affecting man and animals."

Art was also a member of the team at the Department's Orlando, Florida laboratory which received the 1947 Distinguished Service Unit Award

for its contribution to the health and welfare of mankind. He is a very active and interested member of AMCA, and served as its president in 1957. Many AMCA members will also recall with great pleasure the gracious hospitality of both Art and Mrs. Lindquist on the occasion of the annual meeting of the Association in Washington, D. C. in 1958. All members of AMCA are proud and pleased that one of our number has been singled out by so important an organization as the U. S. Department of Agriculture to receive its highest honor.

The **NORTHEASTERN MOSQUITO CONTROL ASSOCIATION** will hold its 1962 annual meeting on Wednesday and Thursday, February 14 and 15, at the Quartermaster Engineering and Research Command in Natick, Mass. The tentative program includes a review of mosquito control methods with particular reference to new developments, by Dr. Arthur Lindquist. Dr. John Lilly will report on some studies of the drift of insecticides from aerial applications. Clyde Barnhardt of the Ft. Belvoir engineering laboratories will discuss the work on development of new types of equipment. Charles F. Foster, Massachusetts Commissioner of Natural Resources will speak on the move to obtain legislation to reduce the hazards of pesticide usage. It is hoped that Dr. Wayland Hayes of the USPHS will have a review of the actual record of pesticide hazards to humans. The meeting will include a special period for mosquito control field workers to discuss their experiences and problems. A banquet and entertainment are planned for Wednesday evening.

The **CALIFORNIA MOSQUITO CONTROL ASSOCIATION** will have its 30th Annual Conference in the Villa Hotel at San Mateo, California on January 29–31, 1962. The program will emphasize research, and will comprise contributions from the different organizations and institutions engaged in mosquito research in California, including the various branches of the University of California, the State Department of Health, the U. S. Department of Agriculture and the U. S. Public Health Service.

REVIEWS AND ABSTRACTS

HELEN LOUISE DURKEE

DURATION OF THE PUPAL STAGE OF *Aedes taeniorhynchus* WITH A DISCUSSION OF THE VELOCITY OF DEVELOPMENT AS A FUNCTION OF TEMPERATURE. By Nielsen, E. T. and Evans, D. G. Oikos 2(2):201–221. 4 tables, 6 figs. 35 refs. (in English) Copenhagen, 1960. Experiments were carried out to determine the duration of the pupal stage of *Aedes taeniorhynchus* at different constant temperatures. By means of techniques described in detail, it was possible to keep the temperature constant within ± 0.02° C. and determine the moment of transformation within an interval of less than 0.3 percent of the shortest duration of the stage. The analysis of the material was carried out as a determination of the regression of the velocity of development against the temperature, the velocity (v) being $\frac{1000}{d}$ where (d) is the duration of hours. The relation between (v) and the temperature (t) is best expressed by the equation

$$v = a + b \cdot c^t$$

which is the same function Krogh found for the effect of temperature on the respiratory metabolism, and which seems to be generally valid for biological processes as a function of temperature. At high temperatures a negative correction has to be added. The correction has a temperature coefficient which may be expressed by the same function but with different values for the parameters. —Authors' summary.

MEDICAL ENTOMOLOGY. By William B. Herms. Fifth Edition, revised by Maurice T. James, New York: The Macmillan Company, pp. XIV + 616, (185 figures) 1961. $12.00. For more than ten years, this reviewer has used Herms' *Medical Entomology* quite successfully as a textbook. During this period, there have been so many major developments in the field of medical entomology that this reviewer looked forward to the new edition of this book with interest and even trepidation. However, it is with considerable satisfaction that this user of the book can say that the revision is most satisfactory; Dr. Maurice James has done an excellent job of an exceedingly difficult task.

The general plan of presentation of preceding editions has been preserved, as Dr. James points out in his introduction, although certain parts have been strengthened by the inclusion of additional keys and more anatomical material. Two additional changes have also been made of which this reviewer definitely approves: the references at the end of each chapter are arranged alphabetically by author, and some of the case histories of the preceding edition have been omitted. There are twenty-one chapters, the first of which defines medical entomology, outlines the training a medical entomologist should have, and gives a brief history of the subject. This is followed by a chapter on how arthropods cause and carry disease, and one on general control methods. After a chapter on arthropod anatomy, Dr. James discusses the medically important arthropods, beginning with cockroaches and beetles. As might be expected, the Diptera receive major attention, there being no less than nine chapters devoted to the various members of this order.

The first edition of this book was entitled *Medical and Veterinary Entomology*. As knowledge progressed, arthropod transmission of human diseases, as opposed to those of other animals, was emphasized in succeeding editions. This procedure has been followed in the present edition, and as a consequence, some of the veterinary aspects of former editions have been eliminated in order to keep the book within reasonable textbook length.

This reviewer was especially interested in the last chapter, "Venomous and Urtical Arthropods' since this is a subject in which he has been interested for many years. It was most gratifying to note such relatively recent subjects included as Stahnke's L-C treatment of scorpion sting, the dangers to some individuals of wasp and bee sting, and loxoscelism, or spider bite necrosis. Although treatment of various conditions is not emphasized—as it should not have been—the writer would have been happier if the successful use of calcium gluconate for the sting of the puss moth caterpiller, *Megalopyge opercularis* (J. E. Smith), had been mentioned.

Only one incidental error has come to this reviewer's attention so far as the use of up-to-date scientific names is concerned. On page 50, the generic name, *Pediculoides,* is used for a group of viviparous mites that occasionally cause dermatitis in man. This name has been replaced by *Pyemotes,* and this newer designation is used on pages 508 and 509 where one species is discussed.

Dr. James is to be congratulated upon an outstanding achievement.—Osmond P. Breland, The University of Texas.

THE INCIDENCE OF ARTHROPOD-BORNE VIRUSES IN A POPULATION OF CULICINE MOSQUITOES IN TONGALAND, UNION OF SOUTH AFRICA (JANUARY, 1956, THROUGH APRIL, 1960). By Worth, C. B., Paterson, H. E., and de Meillon, Botha. Amer. Journ. trop. Med. Hyg. 10(4):583–592. 4 tables, 1 graph, 14 refs. 1961. From January, 1956,

through April, 1960, 171, 210 mosquitoes from Ndumu, Natal, Union of South Africa, were inoculated into mice in 2,325 lots for attempted virus isolation. Fifty-nine recoveries of viruses were effected.

The viral isolates, including nine identified viral types, were recovered from nine species of mosquito. Among these the ones principally concerned were *Aedes* (*Neomelaniconion*) *circumluteolus* (Theo), *Culex* (*Culex*) *univittatus* Theo., *Mansonia* (*Mansonioides*) *africana* (Theo.), and *Mansonia* (*Mansonioides*) *uniformis* (Theo.), in the order named.

The incidence of viral isolates in time and space at Ndumu indicates the possibility of the following assumptions having some basis in fact: (a) host specificity of most of the viruses concerned is not strict among genera of the tribe Culicini; (b) the virus types occurring in the Ndumu region may have undetected local cryptic cycles, their episodic reappearances being accounted for less by foreign reintroduction than by the occasional conjunction of ecological conditions that permits them to enter mosquito cycles detectable by current methods of investigation.—Authors' summary and conclusions.

The Effect of a Residual House-Spraying Campaign in East Africa on Species Balance in the *Anopheles funestus* Group. The Replacement of *A. funestus* Giles by *A. rivulorum* Leeson. By Gillies, M. T. and Smith, A. Bull. ent. Res. 51(2):243–252. 4 refs. 1960. In an inland region of Kenya and Tanganyika, in the course of an experiment in malaria control by means of house spraying with dieldrin, routine catches of mosquitoes resting in artificial outdoor shelters were maintained. During the 18 months of the pre-spraying period, catches in the South Pare district of Tanganyika consisted mainly of the principal vectors: *Anopheles gambiae* Giles, and *A. funestus* Giles, together with small numbers of *A. rivulorum* Leeson. During the three years following the spraying, *A. funestus* disappeared almost completely from the catches, while *A. rivulorum* showed an increase of about seven times above the former level. It is concluded that this change in numbers of *A. rivulorum* was associated with the disappearance of *A. funestus* from breeding sites formerly occupied by both species. Precipitin tests indicated that *A. rivulorum* is mainly zoophilous and had not played any part in malaria transmission.—From author's Summary.

The Breeding Behaviour of the Type Form of *Aedes (Stegomyia) aegypti* (L.) in South-Western Nigeria in Relation to Insecticidal Control. By Surtees, G. Bull ent. Res. 50(4): 681–686. 15 refs. 1960. The type form of *Aedes (Stegomyia) aegypti* (L.) is widespread in south-western Nigeria, breeding in the basins of rotting tree stumps in the coastal mangrove swamp, in holes in living trees in the freshwater swamp zone, and near Lagos and in domestic containers restricted to village clearings in the inland forest zone. Pot surveys were carried out in a rain forest village 50 miles northwest of Lagos and in the capital itself. Baked clay pots holding half a gallon of water were used in each case and were placed in three situations: inside houses, outside but in otherwise sheltered positions, and in fully exposed positions. The degree of larval infestation in both cases was greatest inside houses, but the behaviour pattern was less domestic in Lagos. Larvae were found in pots contaminated with decaying vegetable matter in Lagos but not in the village. Spraying tests indicated that houses and foliage afforded varying degrees of protection to the immature stages of the mosquito. Control of this species is made difficult by the localization of the immature stages in relatively inaccessible sites, and it is suggested that individual treatment of larval habitats by insecticidal pellets before the commencement of the rainy season would ensure a high mortality of first-generation larvae. The toxicity hazard to man is not considered to be great.

A further difficulty involved in the control of the species is that it rapidly develops resistance to an insecticide such as DDT, and naturally occurring resistant populations are known. . . . Finally, the possibility must not be overlooked of the establishment of wild populations in forested areas when a campaign is directed against a domestic mosquito. This may be caused by the development of behaviouristic resistance, in which case oviposition behaviour of the female may be altered, or by naturally occurring tree-hole breeders building up in numbers and becoming the dominant population in the area. Both possibilities must be considered in view of the marked plasticity in breeding behaviour of this species.—Excerpts from author's Discussion, Conclusion, and Summary.

The Biology of Two Species of Mosquito, *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald) Belonging to the Subgenus *Mansonioides* (Diptera, Culicidae). By Laurence, B. R. Bull. ent. Res. 51(3):491–517. 4 tables, 9 figs. 66 refs. 1960. The author sets forth in detail the life histories, habits, and certain environmental responses of *Mansonia africana* and *M. uniformis* in the laboratory, and reviews the literature on investigations in both laboratory and field.

There are 2 small maps, depicting the distribution of the 2 species. *M. africana* is confined to Africa. *M. uniformis* extends southward from 40° N. in east China, through Cambodia, Thailand, Malaya, India, Ceylon and the Seychelle Islands, into Africa; eastward, it extends through Japan, Java, Borneo, New Guinea, into Australia and the Solomon Islands.

In the adult stage, the 2 species differ in the

color and distribution of scales on scutum and legs, and in the structure of the genitalia of the male and female.

Laboratory colonies on which observations were made include *africana* from Uganda and Tanganyika, and *uniformis* from Malaya, Ceylon, and Uganda. Detailed rearing methods are not given, but reference is made to "The breeding of *Taeniorhynchus* (subgenus *Mansonioides*) mosquitoes in the laboratory," by Laurence, B. R. and Smith, S. A. Proc. R. Soc. trop. Med. Hyg. pp. 518–526, 1958.

Both species are stenogamous; they mated in small cubical cages 25 cm. (approx. 10 in.) on a side, and even in test tubes. Males and females fed on a sugar solution from cotton-wool pads, and on fruits such as apples or raisins. Females of both species fed readily on the blood of man, cat, rabbit, or guinea pig. We assume that the blood was taken by bite and not from blood on cotton-wool. Both species fed approximately 24 hours after emergence and usually fed again immediately after oviposition. At 25°–30° C. oviposition occurred about 3 to 5 days after the blood meal. At 25°–30° C. adults of both species emerged 21–31 days after the eggs hatched.

Fertility between the strains of *uniformis* was established in laboratory experiments, although there was noted a reduction in the fertility of crosses between the African strain and those from Malaya and Ceylon. Attempts to cross *africana* and *uniformis* were not successful.

In both species, the actions of the females prior to and during oviposition are recorded. Oviposition responses to different backgrounds of breeding sites and to water pollution are described in interesting detail. *M. uniformis* appeared to have more visual sensitivity and so was more influenced by the types of background offered for oviposition than was *africanus*. This latter species reacted more acutely to organic pollution of the water offered for oviposition.

Both species react similarly to leaf surfaces when ovipositing. A comparison is made of their responses to 3 species of plants, *Salvinia, Lemna,* and *Azolla*.

The feeding and attachment habits of the larvae were explored in the laboratory, and the conclusion reached that both are filter feeders, will feed on the same type of food, and, if food is present, will attach readily to almost any surface containing air. The pupae of both species develop attached to plants. If they are detached one or 2 days before emergence of the adult, up to 51 percent will continue development and adults will emerge.

In conclusion, the author discusses the similarity of the biologies of *africana* and *uniformis,* the partial infertility between the African strain and the Malayan and Ceylon strains of *uniformis,* and the possible competition in Africa of the two species. He suggests that *uniformis* may be a recent invader, although, so far, there is no evidence of its extending its range or taking over at the expense of *africana.*

It is often difficult for the reader to maintain his train of thought, for throughout the text there is an intermingling of original data and interpretation along with laboratory and field reports from literature.

I wish that Dr. Laurence could have had available for comparison and comment Dr. G. J. Burton's five papers on Indian *Mansonioides.* (Indian J. Malar. 13(2–3) 1959, and 14(2–3) 1960; reviewed in *Mosquito News* 21(2):166, June 1961).

Dr. Laurence has a good knowledge of the literature; his familiarity with and interest in the implications and applications of the findings of others, in addition to his own careful research, makes this an excellent contribution—H. L. Trembley Durkee.

The 14th Annual Meeting of the **UTAH MOSQUITO ABATEMENT ASSOCIATION** will be held March 16th and 17th, 1962, in the Union Building, University of Utah, Salt Lake City, Utah. Dr. George Knowlton, Vice President of the Association, Extension Entomologist of Utah State University, is preparing a program which will stress the role of Education in Mosquito Control. Other recent developments in mosquito control also will be discussed. Dr. Archie Hess will be the banquet speaker on the evening of March 16th.

REFERENCES TO LITERATURE OF INTEREST TO MOSQUITO WORKERS AND MALARIOLOGISTS

HELEN SOLLERS

Plant Pest Control Division, Agricultural Research Service, United States Department of Agriculture, Washington 25, D. C.

AEROSOLS

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